NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

Canada

THE UNIVERSITY OF ALBERTA

A TRIAL OF THE 21-AMINOSTEROID U74006F IN A PRIMATE MODEL OF CHRONIC CEREBRAL VASOSPASM

BY

David Earl Steinke

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

FALL 1988

ISBN 0-315-45761-9

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: David Earl Steinke

TITLE OF THESIS: A Trial of the 21-Aminosteroid U74006F in a Primate Model of Chronic Cerebral Vasospasm

DEGREE: Master of Science

YEAR THIS DEGREE GRANTED: 1988

Permission is hereby granted to **THE UNIVERSITY OF ALBERTA LIBRARY** to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

David Steinke, M.D.
Division of Neurosurgery
2D1.02 WMC
8440 - 112 Street
University of Alberta
Edmonton, Alberta
T6G 2B7

June 27, 1988

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled A TRIAL OF THE 21-AMINOSTEROID U74006F IN A PRIMATE MODEL OF CHRONIC CEREBRAL VASOSPASM, submitted by David Earl Steinke in partial fulfilment of the requirements for the degree of Master of Science in Experimental Surgery.

B.K.A. Weir, M.D., supervisor

D.A. Cook, M.A., D.Phil.

D.B. Russell, M.D.

T. Noseworthy, M.D., M.Sc.

M.G.A. Grace, Ph.D.

This thesis is dedicated
to my wife Margaret, and to
my parents, Rita and Jack

Abstract

The efficacy of U74006F in the prophylaxis of chronic cerebral vasospasm (VSP) was evaluated in a randomized, double-blind, placebo-controlled trial. Forty cynomolgous monkeys were divided by restricted randomization into 2 treatment groups with 20 animals receiving U74006F and the remainder the suspension vehicle. Five animals from each treatment group were randomized into subgroups 1, 2 and 3. The animals of subgroup 1, were studied pathologically. Those in subgroup 2 had brain biopsies studied with High Performance Liquid Chromatography (HPLC). Cerebral vessels were removed from the animals of subgroup 3 for studies of endothelium dependent relaxation. The remaining 10 animals supplemented the number studied angiographically. Significant VSP ($p < 0.05$) was detected in the majority of vessels from the clot side (right) of both treatment groups. Electron microscopy results were in agreement with the angiographic data. Animals treated with U74006F showed significantly less VSP than control animals ($p < 0.05$) in the right extradural internal carotid and middle cerebral arteries (MCA). Two animals developed delayed neurologic deficits, one from each group. The infarct of the U74006F animal was smaller than the infarct in the control animal. Although overall changes in phosphagen levels did not reach statistical significance, HPLC analysis of the cortical biopsies did show a mean decrease in the ATP/ADP+AMP ratio of 54% in vehicle treated animals and only 7% in animals receiving U74006F. MCA's from 2 animals were also studied with HPLC. The vessels from the clot side had moderate

angiographic VSP and the ATP/ADP+AMP ratio was reduced by more than 50% compared to the MCA's of the contralateral hemisphere. This model of SAH did not affect relaxation produced by bradykinin, acetylcholine or adenosine triphosphate. There was no significant difference in vessel relaxation between treatment groups. There may be a role for the use of U74006F in the prophylaxis of VSP following SAH.

Key words: Chronic vasospasm, Subarachnoid hemorrhage, Primate model, Endothelium-derived relaxing factor

ACKNOWLEDGEMENTS

I would like to gratefully acknowledge the excellent technical assistance of Edith Schwaldt, Maxine Farr-Jones, Tsilya Gorodetski and Christal Krueger. Without their expertise this study could never have been completed. I would like to thank Geoffrey Hawkins for the special attention he gave to the animals in the vivarium.

I am indebted to Dr. P.J. Lewis for his instruction in surgical technique, to Dr. F. Espinosa for his assistance in preparing pathologic specimens, and to Dr. J.M. Findlay for his welcomed advice and day to day help.

I would like to thank Dr. David Cook for overseeing the pharmacological aspects of this research and Dr. Michael Grace for the statistical analysis and guidance throughout the last year. Dr. T. Noseworthy provided invaluable constructive criticism in proofreading this manuscript.

Arlene MacLean spent many hours deciphering my ambiguous notes and typing this thesis. I greatly appreciate her efforts and the quality of the end result.

Dr. Ed Hall of the Upjohn Company provided much more tha financial support. He was always available to discuss problems and suggest solutions.

I would like to express my thanks to Dr. Bryce Weir for providing me with the opportunity to work in the cerebrovascular laboratory, for his guidance, and for sharing with me his knowledge of experimental design.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE: INTRODUCTION

A: Overview

Nineteen hundred years ago, St. Paul pondered how 'the world was created by the word of God so that what is seen was made out of things which do not appear'. Today this reality continues to confront researchers of medical science. Each day the physician is compelled to treat disorders, the pathophysiology of which remains obscure. Of the afflictions seen by neurosurgeons, subarachnoid hemorrhage (SAH) and the anathema of vasospasm (VSP) have special significance. After successfully clipping an aneurysm, nothing is more disheartening than to witness a patient suffer a devastating stroke secondary to VSP. The inadequate treatment of VSP stems directly from our inability to comprehend its pathogenesis. Despite the intensive efforts of numerous research facilities throughout the world, VSP remains an enigma.

Chronic cerebral VSP may be defined as an exaggerated constriction of cerebral vessels in response to perivascular blood in the subarachnoid space.[187] It may be focal or diffuse. It is demonstrable angiographically, and develops from 4 to 14 days following SAH with a peak incidence between days 6 and 8.[31,112,150,189,190] While VSP has been described in association with other etiologies, aneurysmal SAH remains the foremost precipitating factor.[21,28,77,109,153,197]

Subarachnoid hemorrhage results from a ruptured aneurysm in approximately 75% of cases.[131] The annual incidence of aneurysmal SAH varies from 3.5-25/100,000 population.[131,139,161,186] About 3000 new cases are reported in Canada each year (12/100,000). In the United States there are 28,000 new cases yearly.[30,91,92]

SAH secondary to aneurysm rupture is a lethal disorder with a mortality of up to 20% by 24 hours, and close to 60% within 6 months of its occurrence.[91,107,139,188]

Among survivors of the initial ictus, the principal factors contributing to morbidity and mortality include rebleeding, hydrocephalus, various medical complications and VSP. Chronic cerebral VSP is the most significant.[79,92,145,187,191]

Approximately 40% of patients will develop VSP after aneurysmal SAH and 50% of these will develop a delayed ischemic deficit (DID). Half of the patients suffering a DID die as a result.[187]

The incidence of VSP appears to be independent of aneurysm size or location, patient age, pre-existing hypertension, or intraoperative hypotension.[79] There is however, a close correlation between the severity of VSP and the amount and location of blood in the subarachnoid space. CT scanning has provided us with a means of predicting which patients will develop VSP.[13,34,44,67,99,160,168,171,172] Espinosa and co-workers were able to substantiate this in the primate model utilized in the present study.[36]

Numerous investigators have shown that there is a relationship between decreased cerebral blood flow (CBF) and angiographic VSP.[113,142,172,192,207] Utilizing Positron Emission Tomography (PET) Grubb and colleagues demonstrated a statistically significant decrease in CBF and cerebral oxygen consumption in areas supplied by vessels in spasm.[66] Another study demonstrated that, in 85% of the hemispheres supplied by vasospastic vessels, there was evidence of ischemic damage in the cortical distribution of those vessels.[61]

B: The Pathophysiology of Vasospasm

A majority of authors support the hypothesis that chronic cerebral VSP results from sustained smooth muscle contraction. Several reports have implicated an infiltrative vasculopathy, yet pathological support is lacking. While the exact physiology of smooth muscle contraction is not fully understood, a basic review is essential to any comprehension of the morbid state.

1. Smooth Muscle Contraction:

In the conventional view, the contractile apparatus of smooth muscle is composed of thin (actin-containing) and thick (myosin-containing) filaments. Tension is generated as a result of interaction between myosin cross-bridges and actin. The thin filaments in smooth muscle are abundant and morphologically similar to those in skeletal muscle. These filaments seem to be anchored at one end to amorphous structures called dense bodies. The dense bodies are found both in the cytoplasm and attached to the plasma membrane. The thick filaments have been described and accepted as a component of the ultrastructure of smooth muscle. The detailed morphology of the thick filaments is unresolved. In particular, the manner in which myosin molecules are assembled to form them remains to be established.

The initial step in smooth muscle contraction is an increase in the cytoplasmic concentration of calcium (Ca^{++}). The intracellular Ca^{++} concentration in the resting state is less than 10^{-7} M. With the initiation of smooth muscle contraction the intracellular concentration of Ca^{++} rises 100-fold. This results from opening of Ca^{++} channels in the plasma membrane.[182]

Unlike skeletal muscle where Ca^{++} is recycled between the sarcoplasmic reticulum and the cytoplasm, smooth muscle depends largely upon external sources of Ca^{++}.[23] There are two, and possibly three methods for increasing intracellular Ca^{++}. The first method, membrane depolarization, is referred to as electro-mechanical coupling. The second results from opening of receptor operated channels and is termed pharmaco-mechanical coupling.[15] Mechanically-sensitive Ca^{++} channels, the third possible means of increasing intracellular Ca^{++} may, in fact, account for the myogenic response outlined by Bayliss in 1902. These channels have been described in endothelial cells[103], and additional evidence supports their existence in smooth muscle.[14,98]

The rise in Ca^{++} allows 4 molecules to bind to calmodulin, a regulatory protein, which in turn binds to myosin light chain kinase. This complex acts upon the light chains of myosin molecules to induce phosphorylation. The phosphorylated light chains stimulate actin-myosin interaction, with free Ca^{++} forming bridges between the two molecules. The energy required is provided by the dephosphorylation of adenosine triphosphate (ATP) by a magnesium-dependent adenosine triphosphatase. Deactivation of the system occurs with a decrease in the availability of Ca^{++} and dephosphorylation of myosin light chain by myosin light chain phosphorylase. Although this scheme is oversimplified, it remains the most popular theory of smooth muscle contraction and regulation.[60,76]

Another prominent theory suggests that phosphorylation of the light chain is not involved in the regulatory process. It has been postulated that a regulatory protein called Leiotonin is involved in this alternate system. The important difference between this and the preceding theory is that leiotonin may function independently of myosin

phosphorylation.[32,33,116] Another distinction is that leiotonin is believed to be located on thin filaments and not myosin-linked.[76] With further investigation the exact mechanism of smooth muscle contraction and regulation will be discerned and perhaps this will provide insight into the complexity of VSP.

2. Free Radicals and Lipid Peroxidation

The work of numerous laboratories has implicated a variety of putative mediators in the genesis of chronic cerebral VSP.[160,184,187] Although most authors support a multifactorial etiology of VSP, there is general consensus that oxyhemoglobin (oxyHb) plays a central role in the cascade of events that culminates in decreased vessel caliber.

Following aneurysm rupture blood is propelled into the subarachnoid space and the coagulated red cells are gradually hemolyzed. The concentration of oxyHb increases to peak 7 days post-ictus.[164] OxyHb, a product of erythrocyte breakdown, has been found in vitro and in vivo to be a potent vasoconstrictor. OxyHb is converted to the non-spasmogenic meth-hemoglobin (meth Hb) within 2 weeks. Of the variety of agents implicated in VSP only hemoglobin oxidation follows the time course of VSP that is seen clinically.[49,86,128,148,164,177,187,193]

Assuming that oxyHb acts as the initiator, what are the biochemical steps leading to sustained smooth muscle contraction? To unravel the cascade of events precipitating VSP one must implicate free radicals generated by the oxidation of hemoglobin, and examine the actions of these free radicals on the smooth muscle cell directly, and indirectly via the endothelium.

A free radical is a molecule containing an odd number of electrons and therefore an open bond or half bond, rendering it chemically active.[19,20,114,117,195] If two radicals react with a non-radical, another free radical is generated. These substances are very toxic and accumulating evidence suggests that they mediate tissue damage in a variety of pathological conditions. Disorders in which free radicals may play a role include: myocardial ischemia[20,22,41,58,158], atherosclerosis[198], inflammation[48,81], pancreatitis[134], pulmonary oxygen toxicity[19,176], ischemic damage to the digestive tract[20,62,64,134,135], radiation injury and damage caused by chemotherapeutic agents.[19] This is but a partial list.

With respect to the central nervous system (CNS), free radicals have been implicated in cerebral ischemia[19,24,29,45,97,102,115,118,202,203], and brain and spinal cord injury[68,69,185] Cardinal to the present discussion, is that several authors have suggested that free radicals play a role in the development of VSP.[5,147-149,151,155,156]

With conversion of oxyHb to methHb superoxide anion is released.[117,165,199] Xanthine oxidase is an enzyme that also generates superoxide radicals. It has been found in vascular endothelium and brain capillaries. However, Kim and co-workers have refuted the role of the xanthine oxidase system in the pathogenesis of VSP.[97]

The superoxide released with the oxidation of hemoglobin initiates peroxidation of polyunsaturated fatty acids (PUFAs) in cellular membranes, either directly, or indirectly via the hydroxy radical, singlet oxygen, or the alkoxy radical. By measuring the metabolites of this sequence, several investigators have confirmed that this reaction occurs

in animal models of SAH and in patients with SAH.[5,7,149,155,169] Hemoglobin and other hematin compounds have been shown to catalyze this reaction.[179] Furthermore, lipid peroxidation of biological membranes is a chain reaction, resulting in amplification of the destructive effect.

An intrinsic feature of lipid peroxidation is its dependence upon the availability of iron.[195] Zaleski and Floyd have suggested that regional susceptibility of the brain to oxidative damage may in part be governed by the local endogenous iron content.[205] In a limited number of cases, iron-chelating agents have been more effective than free radical scavengers in preventing lipid peroxidation. In any event, the availability of iron in a dissolving blood clot is unquestioned, thereby setting the stage for free radical induced lipid peroxidation.

Free radicals do more than initiate lipid peroxidation. They also result in non-enzymatic generation of lipoxygenase metabolites. Several of these, 5-hydroperoxy eicosatetraenoic acid (5-HPETE), and leukotrienes C_4 and D_4, for example, cause smooth muscle contraction and have been implicated in chronic cerebral VSP.[42,100,105,133,141] Some have been measured in the CSF of patients with SAH and found to correlate with the presence of VSP.[7,169] Therefore, in addition to lipid peroxidation, free radicals also beget substances that directly cause smooth muscle contraction.

Free radicals may have a destructive effect on smooth muscle cells. Sasaki and Sano have published electron micrographs depicting myonecrotic changes and degeneration of the tunica media of canine basilar arteries subjected to the intracisternal injection of 15-hydroperoxy arachidonic acid (15-HPAA).[149,156] These results are controversial as other authors have been unable to reproduce

them.[35,140] However, a majority of investigators have demonstrated extensive endothelial damage following SAH.[2,26,40,85,106,162,201] This finding has also been demonstrated in the primate model utilized in the present study.[39]

The role of the endothelial cell in VSP has been debated. Sano has suggested that endothelial damage is a direct result of lipid peroxidation. This damage is said to initiate platelet adherence and the production of thromboxane A_2 (TXA_2), a potent vasoconstrictor. The damage is also thought to decrease the synthesis of prostacyclin (PGI_2), a vasodilator. Furthermore, PGI_2 synthesis is inhibited by lipid hydroperoxides.[149] Sasaki et al have shown in their canine model that the synthetic activity of PGI_2 in arteries exposed to subarachnoid blood is decreased 3 and 8 days post-SAH.[154]

The theory of derangement in the regulators of vascular tone has found further support with the discovery of Endothelium-Dependent Relaxation Factor (EDRF) and its purported role in VSP. The vasodilatory effects of several pharmacological agents, including acetylcholine, bradykinin, and adenosine nucleotides, depends in peripheral vessels upon an intact vascular endothelium.[25,52,53] The relaxation produced by these agents is mediated by EDRF. Results from several studies suggest that more than one EDRF may exist. Endothelium-dependent relaxation has been demonstrated in cerebral arteries both in vivo and in vitro[87,146], but these vessels differ significantly from peripheral vessels in terms of endothelium dependent responses. Acetylcholine and ATP cause complicated responses in cerebral arteries with both dilator and constrictor actions. There is no

consensus as to the dependence of either of these responses on the presence of an intact endothelium. Bradykinin, on the other hand, also seems to have a dual effect in cerebral arteries, where an endothelium-dependent relaxation and an endothelium-independent contraction are observed. There does not seem to be any agent which, in cerebral arteries, generates an endothelium-dependent relaxation uncomplicated by other vascular actions.

Synthesis and release of EDRF by the endothelium appears to be associated with a receptor-mediated increase in cytoplasmic Ca^{++}. Peach and colleagues have proposed that the initial source of Ca^{++} is intracellular but extracellular Ca^{++} contributes greatly to the overall response and is vital to the sustained release of EDRF.[87]

The mechanism by which EDRF induces relaxation remains an enigma. However, there is evidence to suggest that EDRF, after diffusing from endothelial cells to the smooth muscle in the tunica media, stimulates guanylate cyclase causing an increase in cyclic guanosine 3',5'-monophosphate (cGMP) levels.[53,136] By unknown mechanisms relaxation ensues. It has been proposed that cGMP-dependent phosphorylation and dephosphorylation of myosin light chain in smooth muscle, may mediate the relaxation.[54,144]

Aggregation of platelets results in the release of a number of substances that stimulate the endothelium to produce EDRF. These include adenosine nucleotides (ATP and ADP), 5-hydroxytryptamine (5-HT), platelet-activating factor (PAF), vasopressin (VP), and thrombin. PGI_2 is synthetized by intact endothelium and prevents platelet aggregation. The net effect of interaction of these substances with viable endothelium is vasodilatation. This in turn tends to wash

away developing thrombus. Conversely, in the presence of significant endothelial damage, there is a different sequence of events. Platelet aggregation is enhanced by exposed collagen and depending on the type of blood vessel, liberated substances such as thrombin, VP, 5-HT, ATP and TXA_2 can directly stimulate the smooth muscle cell producing vasoconstriction.[57,180,181]

Endothelial damage, in concert with the reduction in EDRF and PGI_2, leaves the smooth muscle cells more susceptible to circulating and periarterial spasmogens. In addition to endothelial damage, disruption of interendothelial junctions may be an important means whereby vasoconstrictors gain access to the underlying smooth muscle.[152]

Using a variety of animal models, investigators have recently demonstrated that experimental SAH, and administration of oxyHB can impair endothelium-dependent relaxation,[57,124,126] while methHb has little or no inhibitory effect.[55] Furthermore, Kanamaru et al have confirmed that CSF from SAH patients also inhibits the endothelium-dependent relaxation produced by the calcium ionophore A23187.[88] In cell-free systems, hemoglobin impairs the stimulation of soluble guanylate cyclase.[123] This may be the basis for its blocking action, as the increase in cGMP that normally accompanies the relaxation produced by acetylcholine and A23187, is abolished by hemoglobin.[110]

There are other inhibitors of EDRF activity. Moncada and colleagues have demonstrated that phenidone, BW755C, dithiothreital, hydroquinone and pyrogallol all prevent EDRF production through the formation of superoxide anions.[120] Impairment of endothelium-dependent relaxation has been implicated in the increased vascular tone

accompanying myocardial ischemia and reperfusion. Although the mechanism is unclear, free radicals are thought to play a role.[179]

Accumulating evidence suggests that free radicals derived from the oxydation of hemoglobin, can initiate a cascade of events culminating in VSP. The effect of free radicals on endothelial cells may be of importance. Recently it has been proposed that EDRF may be nitric oxide. Palmer et al have demonstrated that nitric oxide is identical to EDRF in terms of biological activity, stability and susceptibility to a potentiator and an inhibitor.[132] This information may provide new insights into the mechanism of VSP.

C: Prophylaxis and Treatment of Vasospasm

Prevention should take precedence over treatment. However, the obviation of numerous disorders is impossible and physicians are obliged to prescribe various remedies, some without proven efficacy and with potential to harm. The treatment of chronic cerebral VSP following SAH is a case in point. At present there is no screening procedure for aneurysms with sufficiently low morbidity and mortality. Visualization of the vascular tree by Magnetic Resonance has shown promise, but to date, only angiography provides acceptable resolution. Although prevention of VSP remains the objective, treatment of symptomatic VSP is currently more effective.

Innumerable diverse approaches have been applied to both prevention and treatment of chronic cerebral VSP. Attempts have been made to dilate spastic cerebral vessels, improve cerebrovascular rheology and oxygen delivery, remove blood from the basal subarachnoid space, and to increase cerebral blood volume, blood pressure or cardiac output.

No modality, pharmacologic or otherwise, has consistently reversed the angiographic VSP seen one to two weeks following SAH. Some success has been achieved in decreasing the incidence of DID's and improving the clinical condition of patients suffering from symptomatic VSP. Wilkins has extensively surveyed the many approaches utilized in treatment and prophylaxis.[196] To date, the only methods of prevention that show promise are clot removal and the calcium antagonists. With respect to treatment, hypertensive, hypervolemic hemodilution is presently the only means available for the treatment of patients with symptomatic VSP.

1. Clot Removal

Mechanical removal of subarachnoid clot was first suggested 2 to 3 decades ago.[25,143] In the last several years, early removal of subarachnoid blood has been attempted by mechanical and pharmacological methods.

In a primate model, Nosko et al demonstrated, in a blind randomized trial, that clot removal could prevent VSP and DID's.[130] Handa and co-workers extended these results, showing that surgical evacuation of blood clot was effective only if performed within 48 hours of SAH.[73] In a review of 181 patients by Mizukami and co-workers, a decreased incidence of VSP was found if subarachnoid clot could be successfully removed.[119] Taneda, in a nonrandomized study of 239 patients, showed that extensive removal of clot within 48 hours of SAH could reduce the occurrence of DID.[174] Kawakami and Shimamura used external cisternal drainage for at least 14 days following SAH in 21 patients.[96] Although 5 developed symptomatic VSP, all were in good neurological condition by

Most neurosurgeons recognize that extensive mechanical removal of perivascular blood in patients with acute SAH is technically difficult and hazardous. Therefore, interest has grown in the study of intrathecal thrombolytic agents, as a means of eliminating subarachnoid clot, thereby preventing VSP. Several thrombolytic drugs have been employed in animal models and SAH patients.

In a porcine model, Alksne and colleagues prevented post-SAH vasculopathy with a single intrathecal dose of 100 units of plasmin.[3] Preliminary reports of uncontrolled studies from Japan have suggested that continuous ventriculo-cisternal irrigation with solutions containing urokinase may be efficacious in dissolving subarachnoid blood.[157,204]

Investigators are currently studying the use of tissue plasminogen activator (tPA) in a primate model of chronic cerebral VSP. Findlay and co-workers have recently completed a randomized, double-blind, placebo-controlled trial.[42] They were able to demonstrate a statistically significant decrease in the incidance of VSP in treated animals. Pathological examination disclosed dissolution of all clot in the tPA group and no major side effects were encountered. A safety study has been completed and a bilateral clot study is underway. The thrombolytic agents show promise. In conjunction with early surgery to secure the aneurysm, they may provide effective prophylaxis.

2. Calcium Antagonists

Recognizing the central role that Ca^{++} plays in smooth muscle contraction, it was logical to study Ca^{++} entry blockers for the prevention of VSP. The dihydopyridines, nimodipine in particular, have received the most attention. Nimodipine has a high lipid solubility and

in vitro studies have disclosed a selective action on the cerebral vasculature.[74,141] As a means of prophylaxis nimodipine can be administered orally, intravenously, and intrathecally. Results from animal investigations and clinical trials are conflicting, largely due to differences in experimental design.

Oral nimodipine has been used in randomized double-blind, placebo-controlled trials in the primate model.[37,129] No clear beneficial effect on angiographic VSP was observed. Auer and co-workers administered a 2.4×10^{-5} M solution of nimodipine intrathecally to 17 post-SAH patients.[9] Severe VSP was absent on all angiograms taken a mean of 7 days post-hemorrhage while DID's occurred in 2 patients. In a more recent study, Auer demonstrated angiographic cerebral vasodilation in 9 of 12 post-operative aneurysm patients following a 0.2 mg cisternal injection of nimodipine.[10] Work by Lewis and colleagues, using intrathecal nimodipine in an established randomized, double-blind, placebo-controlled primate model, failed to substantiate Auer's uncontrolled observations.[104]

Two multicenter, randomized, double-blind, placebo-controlled trials of oral nimodipine have been recently completed. In the study of Allen et al, the incidence of DID's ascribed to VSP alone was reduced in the treatment group.[4] No comment could be made with respect to angiographic VSP, as cerebral angiography was not performed consistently. The second trial was conducted on poor grade aneurysm patients. Nimodipine treatment was associated with a better overall outcome, and DID's from VSP were significantly less frequent in the treatment group.[138] In this study angiograms were performed routinely

on the average of 8 days post-SAH. No significant difference was seen in vessel caliber between treatment and placebo groups.

It has been suggested that nimodipine may have a direct cerebral protective effect. Work from experimental focal ischemia supports this.[69] While promising, the role of nimodipine in VSP prophylaxis is still evolving.

The success of nimodipine has led to investigation of other calcium channel blockers. Nicardipine, a monohydrochloride salt of the dihydropyridine series, exhibits preferential activity in cerebrovascular smooth muscle. It has been shown to reverse experimental vasoconstriction and prevent ischemic damage.[50,65] It has been used for the treatment of VSP in Japan, although results were inconclusive because of the small number of patients studied.[77] In a recent dose-escalation study of intravenous nicardipine, the incidence of angiographic VSP and cerebral ischemia was less in patients receiving the higher doses.[46] In another study by the same authors, it was suggested that the incidence of VSP among patients given antifibrinolytic drugs, could be lowered by the concomitant use of nicardipine.[12] A multicenter, randomized, double-blind trial is planned.

3. Hypertensive, Hypervolemic Hemodilution

Once symptomatic VSP has occurred, various measures have been employed to counteract it. In patients with SAH, attempts at dilating vasospastic arteries have been unsuccessful. Most efforts are presently extended towards improving CBF and oxygen delivery. Several studies have suggested that patients with SAH have a low total blood volume and are more susceptible to effects of VSP.[108,163] Hypovolemia is common following SAH and results in increased blood viscosity. Using

combinations of vasopressors, colloid and crystalloid volume expanders, phlebotomy and mannitol, therapy is directed at lowering blood viscosity and inducing hypertensive, hypervolemic hemodilution. This has been shown to be successful in reversing ischemic symptoms.[89,173,200] However, there are no randomized clinical trials to support or refute this approach.

4. Inhibitors of Lipid Peroxidation

If lipid peroxides generated from free radical reactions have a central role in VSP, it must be shown that inhibitors of lipid peroxidation are effective in the prevention or amelioration of VSP. Normally, endogenous biological defense mechanisms limit damage caused by toxic oxygen species. In endothelial cells, superoxide dismutase (SOD) metabolizes superoxide anion, producing hydrogen peroxide (H_2O_2) and oxygen (O_2). Catalase converts H_2O_2 to O_2 and water (H_2O), and the glutathione redox cycle catabolizes H_2O_2 at the expense of reduced glutathione.[166] Of note, SOD activity appears markedly decreased in the CSF of patients with symptomatic VSP.[148] The patients with low SOD activity also had high CSF concentrations of lipid peroxides.

Lipid peroxidation is a chain reaction which terminates when lipid radicals react to form stable products, or when they react with scavenger molecules such as vitamin E or sulfhydryl groups. Vitamin E (α-tocopheral) is a fat-soluble vitamin that concentrates in the hydrophobic interior of membranes. It donates a hydrogen ion to a peroxy radical and so interferes with the chain reaction of lipid

peroxidation.[71] Even with the remaining unpaired electron, vitamin E is unreactive and degrades harmlessly.

Peroxidation of cell membranes may be inhibited in two ways. The initiator, oxyHb, can be removed (this method been discussed under Clot Removal), or free radical scavengers can be administered prophylactically.

use of steroids such as methylprednisolone and dexamethasone is a time-honored neurosurgical practise. High dose methylprednisolone therapy was used in a double-hemorrhage canine model of experimental SAH.[26] The results suggested a reduction, and in some cases prevention, of angiographic spasm. Pathologic and pharmacologic studies revealed that treated specimens looked and reacted better than those receiving no treatment. The authors concluded that the 'anti-inflammatory' activity of methylprednisolone was responsible for prevention of VSP. In a second study the same investigators used high dose methylprednisolone in 21 patients felt to be at high risk for VSP after SAH. Outcome was better in the treatment group, but deaths secondary to VSP were not significantly different. Regular followup angiograms were not performed and therefore no conclusions can be drawn about angiographic VSP.[27]

In models of cerebral ischemia, the chemiluminescence technique has been utilized to demonstrate free radical reactions. Using the same technique steroids have been shown to suppress these reactions.[118,167] One could thus, just as easily conclude that the beneficial effect of methylprednisolone demonstrated by Chyatte et al was secondary to its effect on free radical reactions.

Nizofenone is a free radical scavenger that has been studied in a cooperative double-blind clinical trial. Although it did not prevent angiographic VSP, it was superior to placebo with respect to neurological outcome following SAH.[149]

Asano et al used another inhibitor of lipid peroxidation in their canine model of experimental SAH. AVS (1,2-bis(nicotinamide)-propane) was given by continuous intravenous infusion and dramatically suppressed the occurrence of chronic VSP in a dose-dependent manner.[6]

A novel series of 21 aminosteriods has recently been developed by the Upjohn Company. These agents have no glucocorticoid receptor-binding activity. Several are potent inhibitors of iron-dependent lipid peroxidation. One such compound, U74006F, is particularly effective in brain homogenates exposed to increasing concentrations of iron.[17]

In an experimental head injury model U74006F was shown to enhance early neurological recovery and survival.[70] It has been demonstrated to retard the development of post-spinal cord injury ischemia.[68] It is protective against post-ischemic mortality and neuronal necrosis in a gerbil model of cerebral ischemia.[69] U74006F is the subject of the present study.

D: Summary of the Present Study

This study utilized an established primate model of chronic cerebral VSP that closely approximates the human condition. It investigated several aspects of SAH-induced VSP.

Although our understanding of the pathophysiology of VSP remains incomplete, free radicals and lipid peroxidation may be of central importance. The 21-aminosteroid U74006F is a potent inhibitor of iron-dependent lipid peroxidation. The dosage chosen for the present study was determined from data of other investigations. Prior to this study, the treatment drug had not been used in an accepted model of SAH, nor had it been used in primates.

Cerebral VSP was defined by angiographic and pathologic criteria. Angiograms were carried out in a standardized fashion. Mean vessel diameter was calculated on Day 0 and 7 days post-SAH induction. VSP was said to be present if vessel caliber had decreased by more than 10%. Scanning and transmission electron microscopy were used to corroborate the angiographic data. In addition, these modalities were used to look for pathologic changes suggestive of lipid peroxidation.

Cerebral ischemia results when CBF is inadequate to meet tissue demands. Compromised CBF results in a shift towards anaerobic metabolism and eventually loss of high energy phosphate compounds. VSP, if severe enough, results in cerebral infarction and this manifests clinically as a DID. High energy phosphates supply neurons with the energy to maintain their metabolic machinery. As ischemia progresses, the supply of ATP declines, and its metabolites ADP, AMP and inorganic phosphate (Pi) increase.[8,82,159] The ATP/ADP+AMP ratio is therefore a good index of ischemia. It provides a means of recognizing ischemia prior to infarction.

Using a cerebral ischemia model and high performance liquid chromatography (HPLC), Morimoto and co-workers demonstrated a

decrease in ATP and increase in lower energy phosphates.[121] HPLC is an invasive technique that gives accurate, quantifiable data. It was used in the present study to compare the ATP/ADP+AMP ratio in areas of cortex supplied by vasospastic and nonspastic vessels.

Adventitial stomas have been identified in cerebral vessels. These apertures appear to connect the subarachnoid and intra-adventitial spaces. CSF may nourish the vessel wall through these pathways.[38,206] In SAH the stomas are blocked by erythrocytes and the intra-adventitial spaces are obliterated by fibrosis. This may alter the metabolism of the underlying smooth muscle, rendering it more susceptible to vasogenic substances. Several investigators have demonstrated that hypoxic vessels are more susceptible to various agonists, including hemoglobin.[125,183] Perhaps this results from the facilitation of transmembrane influx of extracellular Ca^{++}.[125] In the present study, biopsies of MCAs were taken to investigate the energy status of vessels in spasm and the effectiveness of U74006F at this level.

Endothelial cells have a strong influence on the metabolism and activity of underlying smooth muscle. Endothelial damage occurs following SAH and may be induced by free radicals. The 21-aminosteroid U74006F inhibits lipid peroxidation and may protect the endothelium. Recently it has been demonstrated that endothelium-dependent relaxation is adversely affected by SAH. In the present study, the effect of SAH on endothelium-dependent relaxation was evaluated, and the ability of U74006F to preserve EDRF activity was ascertained.

E. Objectives and Hypothesis:

OBJECTIVES

1. Primary Objective

   The primary objective of this investigation was to study the effect of the 21-aminosteroid U74006F on chronic cerebral vasospasm in the primate model.

2. Secondary Objectives

   a) As an index of ischemia High Pressure Liquid Chromatography (HPLC) was used to examine high energy phosphates in cerebral vessels and biopsies of cerebral cortex.

   b) If ischemia was present and measurable by HPLC, the effect of U74006F on its severity was quantified.

   c) The model was used to assess the reported adverse effect of SAH on endothelium dependent relaxation and establish whether this response could be inhibited by U74006F.

HYPOTHESIS

1. Primary Hypothesis

   Chronic cerebral vasospasm is as likely to occur in animals given treatment A (U74006F) as given treatment B (vehicle).

2. Secondary Hypothesis

   a) There is no difference in the ATP/ADP+AMP ratio in cerebral vessels and cortical biopsies between animals given treatment A (U74006F) vs treatment B (vehicle).

   b) There is no difference in the degree of endothelium-dependent relaxation in the vessels from animals given treatment A (U74006F) vs treatment B (vehicle).

CHAPTER TWO: MATERIALS AND METHODS

A: Randomization and Blinding

This study was designed as a randomized, double-blind, placebo-controlled trial. Forty female cynomolgous monkeys (Macaca Fascicularis, Charles River Primate Research Corp., Port Washington, New York) weighing between 2.5 and 4.5 kg, were assigned by restricted randomization into 2 groups of 20. The treatment groups were further subdivided at random with 5 animals from each placed into subgroups 1, 2 and 3. The animals of subgroup 1 underwent pathological examination post-mortem while those in subgroup 2 had biopsies taken and studied with HPLC. Subgroup 3 animals had cerebral arteries removed for investigation of endothelium-dependent relaxation. The remaining animals were utilized to supplement the number studied angiographically.

The suspension vehicle of U74006F was administered for control purposes. Both U74006F and the vehicle were clear and colorless. They were shipped from the Upjohn Company (CNS Diseases Research Unit, Kalamazoo, Michigan) in identical 100 ml bottles. An independent observer labelled each bottle with a number (1-40) and the appropriate subgroup, 1,2,3, assigned to the animal to be tested. Randomization to study groups was therefore blinded to the surgeon, the observer making radiological and pathological measurements, the investigator conducting pharmacological studies, and the statistician analyzing the data.

B: Day 0: Baseline Evaluation and Cerebral Angiography

The animals were sedated with ketamine hydrochloride 6-10 mg/kg IM and then weighed and intubated. All monkeys were ventilated with a 2:1 mixture of $N_2O:O_2$ administered by a variable phase animal respirator (Harvard Apparatus, Inc., Millis, Massachusetts). The ventilatory rate was adjusted in accordance with arterial blood gas measurement to maintain a $PaCO_2$ of close to 40 mmHg. Animals were paralyzed with gallamine, 2 mg/kg IV, and fentanyl, 1 mcg/kg IV, was administered for analgesia. Procaine penicillin, 100,000 IU/kg IM, was given prophylactically. Body temperature was maintained at 37 ° C by heating pad and monitored with a rectal thermometer (Tele-thermometer; Yellow Springs Instrument Co., Yellow Springs, Ohio). The femoral artery on either side was dissected under magnification and catheterized with a 5 French radiopaque, sigmoid tip, polyethylene catheter. Five ml of blood was drawn and allowed to clot for later use in creation of the experimental SAH. The catheter was advanced with fluoroscopic guidance into the communis artery, from which both common carotids arise in the cynomolgous monkey. It was connected via a three-way stopcock to a pressure transducer for monitoring of blood pressure and heart rate (Stratham P23dB pressure transducer; Stratham Instrument Co., Oxnard, California). Blood pressure and heart rate were recorded continuously on a Beckman Dynograph R611 eight channel recorder. Then a single arterial phase, anteroposterior cerebral angiogram was obtained. Iothalamate meglumine (10-12 ml) was injected at 300 psi via a Cordis Injector (Cordis Corp., Miami, Florida). Exposure factors were kept constant and a radiopaque magnification standard was utilized for correction to constant magnification.

C: Operative Procedure: Experimental SAH

Upon completion of baseline angiography, the anesthetic was supplemented with sodium pentobarbital, 26 mg/kg IV and additional gallamine where necessary. All animals underwent a right frontotemporal craniotomy, centered at the pterion. To facilitate brain retraction, the ventilatory rate was increased for the duration of the surgical procedure, lowering the $PaCO_2$ to between 25 and 35 mmHg.

With the aid of the operating microscope the arachnoidal cisterns were opened, exposing the intradural carotid artery, the precommunicating anterior cerebral, and the sphenoidal segment of the middle cerebral artery (MCA). After opening Liliequist's membrane, the posterior communicating and ipsilateral posterior cerebral arteries were exposed. Four to 5 mls of clotted blood were placed in contact with the exposed cerebral vessels. The dura mater was closed in a water tight fashion. A piece of Gelfoam (Upjohn Co. of Canada, Don Mills, Ont.) was placed over the dural incision. Then the wound was closed in layers. A venous cutdown was performed in the posterior calf region and an indwelling catheter inserted. The femoral catheter, used throughout the procedure for arterial access and blood pressure monitoring, was withdrawn, and the femoral artery ligated. The wound was closed with a running polyethylene monofilament suture.

Following completion of the procedure, prostigmine 0.07 mg/kg IV and atropine 0.02 mg/kg IV were given to reverse paralysis and the animals were ventilated with 100% oxygen until breathing spontaneously. They were extubated with return of the gag reflex and then returned to the vivarium.

D: Days 1 to 6: Drug Administration and Clinical Observation

All animals began treatment 20 hours after the induction of experimental SAH. Twenty monkeys received U74006F intravenously, 1.5 mg/kg. The other 20 animals received the corresponding volume of the vehicle. Heparin-lock solution was injected into the indwelling venous catheter following each infusion to maintain patency. This procedure was carried out every 8 hours for the next 6 days. Throughout this period the monkeys were monitored closely for the development of a delayed ischemic deficit (DID). An animal was considered to have a DID if left-sided neurological signs developed more than 48 hours post-SAH. A magnetic resonance study (MRI) or CT scan was obtained in the event of a DID. The MRI scans were performed on a Bruker BNT 100 N imaging system, obtaining proton images at 100 Mhz. Conventional CT images were acquired on a Picker SS03 CT scanner. Animals were sedated for all scans with sodium pentobarbital, titrated individually to achieve light anaesthesia. They were intubated in order to maintain airway patency, which is at risk during the period of neck flexion required for MRI scanning.

E: Day 7: Repeat Cerebral Angiography and Sacrifice

At Day 7 all animals were reanesthetized and had cerebral angiography repeated. Ventilation was adjusted in accordance with arterial blood gas analysis to maintain a $PaCO_2$ of 40 mmHg. Upon completion of angiography the animals were sacrificed in a manner dependent upon their subgroup number.

Animals in subgroup 1 underwent perfusion-fixation. The chest was opened via a midline sternotomy and the left ventricle cannulated.

After cross[illegible]ding aorta 500 ml of normal saline was infused at a pressure of 110 mmHg, thus clearing the vessels of blood. Tissues were fixed with a 500 ml solution of 2% glutaraldehyde and 2% formaldehyde in Millonig's buffer (0.12 M, pH 7.4 at 4° C). The calvarium was then removed and the brain extracted. Utilizing the magnification afforded by the operating microscope, the vessels of the Circle of Willis were dissected free and placed into the glutaraldehyde-formaldehyde solution. The brain was immersed in formalin.

Following angiography the animals of subgroup 2 underwent a left parietal craniotomy. Then the previously performed right craniotomy was extended posteriorly and the dura opened bilaterally. A biopsy forcep was immersed in liquid nitrogen for 1-2 minutes and biopsies were taken of right and left parietal cortex. The forcep was refrozen between biopsies. The tissues were immediately submerged in liquid nitrogen until frozen. The specimens were then placed in a microcentrifuge tube containing 300 mcl of 0.4M perchloric acid. The combination was homogenized briefly and refrozen in liquid nitrogen. In two animals an arachnoid dissection was carried out on the left side freeing the MCA from the carotid bifurcation to the cortical surface. The remaining blood clot was then evacuated from the right side and the M1 segments of both MCA's were harvested in a manner similar to the cortical biopsies. The bleeding that resulted from avulsion of the MCA was quickly controlled with suction and bipolar cautery.

Different methods of acquiring the arterial specimens were investigated. The first technique involved coagulation of the carotid artery as far proximal to the bifurcation as possible. The M1 segment of

the MCA was cut distal to the coagulation site and extracted with the frozen biopsy forceps. HPLC analysis of the arteries obtained in this manner revealed an absence of measurable PCr in all specimens, and little ATP in the majority. This was believed to be due to effects of coagulation and the length of time required to freeze the specimen.

A second method utilized a vascular clip in place of the cautery. For technical reasons this also required too much time.

The third technique involved freezing of the entire brain and basal vessels by pouring liquid nitrogen over the exposed dura. A styrofoam cup was placed within the craniotomy and filled with the liquid nitrogen. All monkeys were maintained in a deep level of anesthesia prior to in vivo freezing. Although this method is known to be excellent for freezing the brain, it prohibited removal of the MCA unless thawing occurred. Phosphagens were detectable in only one specimen studied in this manner.

A fourth method involved using a syringe and catheter system to locally apply the liquid nitrogen to the exposed MCA. This was attempted simultaneously on both sides of the brain. The brain became extremely swollen and herniated through the craniotomies making acquisition of the arterial specimens impossible.

The animals of subgroup 3 were sacrificed by exsanguination. Following an intravenous dose of pentobarbital (26 mg/kg), a midline sternotomy was performed. A cannula was inserted into the left ventricle and the descending aorta cross-clamped. Intra-arterial perfusion was carried out with 500 ml of normal saline at 110 mmHg pressure. This eliminated intravascular blood. The calvarium was removed and the brain extracted. The entire circle of Willis was

dissected free under magnification. The right and left MCA's and the basilar artery (BASA) were placed in oxygenated Kreb's bicarbonate solution (Na^+, 132 mM; K, 5.9 mM; Ca^{2+}, 2.5 mM; Mg^{2+}, 1.2 mM; Cl^-, 122.7 mM; HCO_3^-, 25 mM; SO_4^{2-}, 1.2 mM; $H_2PO_4^-$, 1.2 mM; and dextrose, 11 mM). Both ACA's were placed into the glutaraldehyde-formaldehyde solution.

F: Data Measurements and Analysis

1. Radiology

Radiological assessment was carried out with the aid of a calibrated optical micrometer. The following arteries were measured bilaterally: extradural internal carotid (C3-ICA), intradural internal carotid (C4-ICA), precommunicating anterior cerebral (A1-ACA), sphenoidal segment of the middle cerebral (MCA), and the distal azygous anterior cerebral (A2-ACA). Each vessel was measured 4 times and mean values were determined. Vasospasm was defined as a reduction in the angiographic vessel caliber of greater than 10% of baseline value. A decrease of 11-30% was graded as mild, 31-50% moderate, and greater than 50% severe.

2. Pathology

Pathological specimens were prepared as follows. Brains were kept in formalin for one week prior to sectioning. Sagittal sections were then made and examined for gross evidence of infarction. The specimens for light microscopy were dehydrated through a graded ethyl alcohol series and cleared in xylene. Tissues were transferred to an incubator (58 ° C)

and were moved through 2 changes of paraffin (Tissue Prep embedding pellets; Fisher Scientific) at 56.5 ° C. The blocks were sectioned at 8 μm on a steel knife in a rotary microtome, floated onto albuminized slides, and allowed to dry overnight at 40 ° C. The slides were stained with Harris' hematoxylin and counterstained with alcoholic eosin Y.

The cerebral vessels were kept in the glutaraldehyde-formaldehyde solution for several days and then divided into segments for scanning and transmission electron microscopy (SEM and TEM respectively). All specimens were washed for 45 minutes through 3 changes of Millonig's buffer (0.13 M), and were re-fixed in 1% osmium tetroxide in Millonig buffer at 0.07 M. They were then washed for 30 minutes through changes of distilled water and dehydrated through a graded series of ethyl alcohol.

SEM samples were transferred in absolute alcohol to a CO_2 critical point dryer (Seevac Inc., Pittsburgh, Pennsylvania) to be dried. They were mounted in aluminum stubs and sputter coated with gold (model S150B; Edwards; Crawley, West Sussex, England). They were examined in a scanning electron microscope at 25 KV (Phillips model 505, Gloeilampenfabrieken, Eindhoven, The Netherlands).

TEM samples were transferred in absolute alcohol through 3 changes of propylene oxide for 30 minutes. They were fixed in propylene oxide: aryldite (CY212) epoxy resin; 1:1, for 4 hours and then embedded in resin blocks. They were allowed to cure at room temperature for 24 hours and were then polymerized for 48 hours at 60 ° C. Sections were cut on an ultra microtome (Reichert-Jang Ultracut) and were mounted on 300 mesh copper grids. Specimens were counter-stained with uranyl

acetate and lead citrate. They were examined in a transmission electron microscope at 80 KV (Phillips model 410).

3. High Performance Liquid Chromatography

HPLC was utilized to study the high energy phosphates in the biopsy specimens. Following acquisition, the phosphagens were extracted from the frozen cortical and arterial samples. Tubes containing the biopsies were placed into an ice bucket and allowed to thaw briefly. The centrifuge head which had previously been cooled in a freezer at -20 ° C, was reattached to the centrifuge. The specimens were spun at 14,000 x g for 30-60 seconds, until all particulate matter had migrated to the bottom. The aqueous, or middle phase, was drawn up in a micropipette and placed into separate labeled tubes. The pellet, in its entirety, was scraped into a Nunc tube and placed into liquid nitrogen until the time of protein analysis. Ten μl of Bromothymal Blue were added to each tube containing the aqueous phase. Alanine (3.1 ml) and Freon (9.4 ml) were combined and 300 μl of this solution were added to each tube. The samples were re-centrifuged for 60 seconds. This resulted in a 3 layer separation. The aqueous layer was removed and then frozen until the time of analysis.

The specimens were examined using a gradient elution method of ion-exchange chromatography. This technique employed a variable UV visible spectrophotometer (Waters Model 481, Waters Chromatography Division, Mississauga, Ontario). The HPLC set up program, GRADSTAR, was used to establish a flat baseline. Prior to changing to the analysis program GRADPROG, it was mandatory that the baseline was flat for a minimum of 10 minutes. GRADPROG is based upon a 3-stage

gradient of varying concentrations of a mobile phase. The mobile phase was made up of Solution A (15 μ M $(NH_4)_2PO_4$) and Solution B (0.35 $(NH_4)_2PO_4$ with 0.65 M KCl). The wavelength of the spectrophotometer was changed from 224 nm to 241 nm at 16-18 minutes, shortly after the phosphocreatine (PCr) peak. The adenosine monophosphate (AMP) peak appeared regularly at 5-6 minutes, PCr at 12-14 minutes, adenosine diphosphate (ADP) at 19-20 minutes, and adenosine triphosphate at 32-33 minutes.

The quantity of protein in each biopsy was determined with a commercially available Bio-Rad assay (Bio-Rad Laboratory, Mississauga, Ontario). The pellets were thawed and placed into microcentrifuge tubes. They were spun at 14,000 x g for 2 minutes and then the supernatant was drawn off and discarded. Three hundred μl of 0.4 M perchloric acid were added to each tube containing a pellet and the combination was mixed together with a small pestle. After centrifugation at 14,000 x g for 2 minutes, the supernatant was discarded. This step was repeated once and then 300 μl of acetone were added to the pellet, mixed as before, and centrifuged at 14,000 x g for 2 minutes. The brown colored aqueous was removed with a micropipette. The acetone washing was repeated until the supernatant appeared colorless. The pellet was allowed to dry in a low temperature oven for 5 minutes. Then 500 μl of 1N sodium hydroxide (NaOH) were added to the tube and mixed with the pellet. The tubes were placed into an incubator until the protein dissolved. Those with remaining particulate matter were heated in a water bath at 50 °C for 30-60 minutes. Prior to analysis with the spectrophotometer, a 100 μl aliquot was combined with 5 ml of the dye reagent.

The protein standard consisted of bovine serum albumin and was prepared by adding 20 ml of 1N NaOH to the lyophilized protein to provide a 1.44 mg/ml solution. The concentrated dye reagent was diluted 1:4 with HPLC water and filtered prior to use.

Protein analysis was performed with a Gilford 250 spectrophotometer. The wavelength was set at 595 nm and the slit width at 0.1 nm. Five ml of diluted dye reagent were added to each standard tube and the absorbance measured. A standard curve was then constructed. The unknowns were treated in the same manner and the absorbance of each was used to extrapolate protein values from the standard curve.

4. Endothelium Dependent Relaxation

The MCA's and BASA's were cut into 3 equal sized ring preparations and suspended via 2 stainless steel wires in an organ bath of 10 ml working volume. The organ bath contained Krebs bicarbonate solution, gased with 95% O_2 and 5% CO_2. The resting tension was adjusted to 1 gm and the preparations were allowed to equilibrate at 37 ° C for 90 minutes. Then the vessels were contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 10^{-6} or 5 x 10^{-6} M). Relaxant responses were obtained for bradykinin (BKN; 10^{-10} to 10^{-6} M), acetylcholine (ACH; 10^{-8} to 10^{-4} M), and adenosine triphosphate (ATP; 10^{-8} to 10^{-4} M). This permitted assessment of endothelium-dependent relaxation. Papaverine (10^{-8} to 10^{-4}) was used to examine the endothelium independent response. In all preparations care was taken to ensure that the endothelium was preserved. The dose response curves were

obtained using Grass FT 03 strain gauges connected to a Grass Model 7D polygraph (Grass Instrument Co., Quincy, Mass.)

After several days in the glutaraldehyde-formaldehyde solution, the ACAs were prepared in the manner mentioned previously for SEM. The luminal surface of each specimen was examined under high magnification to ascertain the presence or absence of the endothelium.

5. Statistical Analysis

All data were coded, entered into a computer, and edited. Data for change in angiographic vessel caliber within treatment groups between days 0 and 7 were compared by a paired t-test. Intergroup comparisons were made with a t-test for unpaired variables, and chi-square analysis with Yate's correction where appropriate. An analysis of covariance was used to adjust for any differences that might have occurred at baseline. The HPLC data were compared in a similar fashion. An analysis of variance followed by Scheffe's test was used for multiple comparisons of contractile and vasodilatory responses. The level of significance for all tests of comparison was $p < 0.05$, unless otherwise specified.

The protocol for this study was evaluated and approved by the Animal Ethics Review Committee of the University of Alberta. Care and surgery of the animals were performed according to the standards of the Canadian Council on Animal Care.

CHAPTER THREE: RESULTS

A: Baseline Data

Measurements of body weight, mean arterial blood pressure (MABP), heart rate and $PaCO_2$, from control and U74006F animals, did not differ significantly at baseline evaluation. There was no significant change in any of these indices within each group between Day 0 and Day 7 (Table 1).

B: Clinical Status

One animal died following angiography. The death was attributed to an aortic dissection, and post-mortem examination confirmed the presence of a large retroperitoneal hematoma. A craniotomy was not performed and the animal had not started treatment. Therefore, it was not entered into analysis with the remainder of the animals, which were all in excellent condition following angiography and craniotomy.

Two animals developed a DID. Monkey 17, a vehicle treated animal, developed a left hemiparesis on Day 4 post-SAH (Figure 1). The arm and leg were affected equally. This deficit was accompanied by either a left homonymous hemianopia or left hemispatial neglect. Sensation also appeared to be decreased on the left side. Mild left facial weakness was noted. The DID became more pronounced on Day 5 and then stabilized until sacrifice at Day 7. A CT scan performed on Day 5 displayed a large cerebral infarction, 2x3 cm, in the distribution of the right MCA (Figure 2). This was confirmed by pathologic examination. The second DID occurred in a treatment animal on Day 3 post-SAH. This was characterized by a mild left hemiparesis, primarily affecting the arm,

Table I

Measurements of Physiologic Parameters and Angiographic Vessel Caliber (x ± SD)

	Day 0 Pre-SAH		Day 7 Post-SAH	
	Placebo n=20	U74006F n=20	Placebo n=20	U74006F n=20
Body wt. (kg)	3.3 ± 0.4	3.4 ± 0.5	3.1 ± 0.4	3.3 ± 0.4
MABP (mmHg)	92 ± 8	93 ± 6	92 ± 5	93 ± 6
HR (min^{-1})	133 ± 11	138 ± 8	137 ± 6	137 ± 6
$PaCO_2$ (mmHg)	37 ± 4	40 ± 3	38 ± 4	40 ± 4
Vessel Caliber (mm)				
C3-ICA R	1.36 ± 0.18	1.20 ± 0.20	0.88 ± 0.32	1.05 ± 0.16
L	1.28 ± 0.14	1.19 ± 0.24	1.22 ± 0.15	1.23 ± 0.15
C4-ICA R	0.93 ± 0.14	0.84 ± 0.12	0.61 ± 0.26	0.68 ± 0.16
L	0.89 ± 0.14	0.84 ± 0.12	0.84 ± 0.12	0.85 ± 0.08
A1-ACA R	0.55 ± 0.13	0.45 ± 0.11	0.36 ± 0.15	0.36 ± 0.11
L	0.55 ± 0.14	0.56 ± 0.11	0.53 ± 0.16	0.58 ± 0.07
MCA R	0.66 ± 0.10	0.61 ± 0.11	0.33 ± 0.10	0.44 ± 0.14
L	0.65 ± 0.10	0.61 ± 0.12	0.62 ± 0.09	0.60 ± 0.09
A2-ACA	0.64 ± 0.11	0.64 ± 0.11	0.57 ± 0.09	0.63 ± 0.14

MABP = mean arterial blood pressure
C3-ICA = extradural internal carotid artery
C4-ICA = intradural internal carotid artery
A1-ACA = precommunicating segment of anterior cerebral artery
MCA = middle cerebral artery
A2-ACA = distal azygous anterior cerebral artery
R = right - clot side
L = left - non-clot side

Figure 1

Monkey 17 displaying left hemiparesis that developed on Day 4 post-SAH. By Day 5 the DID became more pronounced and then stabilized until sacrifice at Day 7.

Figure 2

A: CT scan of Monkey 17, placebo animal, showing infarction in the distribution of the right (clot side) middle cerebral artery.

B: Day 7 angiogram of the same monkey showing severe VSP of the right (clot side) vessels.

The deficit remained unchanged until sacrifice at Day 7. A MRI study was performed on Day 4 and a CT scan on Day 5. Both demonstrated small ([illegible] cm^2) cerebral infarction, in the distribution of the right MCA (Figure 3). The pathology confirmed the radiological studies.

C: Extent of Cerebral Vasospasm

The mean angiographic vessel caliber measurements, in millimeters, together with the standard deviations are given in Table 1. Significant VSP ($p < 0.05$) was detected in the following arteries of the U74006F animals: right C3-ICA, right C4-ICA, right ACA and right MCA. In the vehicle treated animals, significant VSP ($p < 0.05$) was detected in the right C3-ICA, right C4-ICA, right ACA and right MCA. No significant changes were noted in the left-sided vessels of either group as a whole, although 2 animals had mild VSP in the left A1-ACA and one, mild VSP was detected in the left MCA. Both were placebo treatec animals.

Intergroup analysis of vessel caliber at Day 0, revealed a significant difference ($p < 0.05$) in the right C3-ICA and right MCA of U74006F animals compared to those treated with vehicle. In both instances the U74006F vessels were smaller. When comparing U74006F to vehicle treated animals at Day 7, adjusting for Day 0 values, there was a significant difference ($p < 0.05$) in the extent of VSP in both right C3-ICA and right MCA. This difference resulted from a greater degree of VSP in the vehicle animals relative to the U74006F group. The right MCA data is displayed in Table 2, and representative angiograms are seen in Figures 4 and 5.

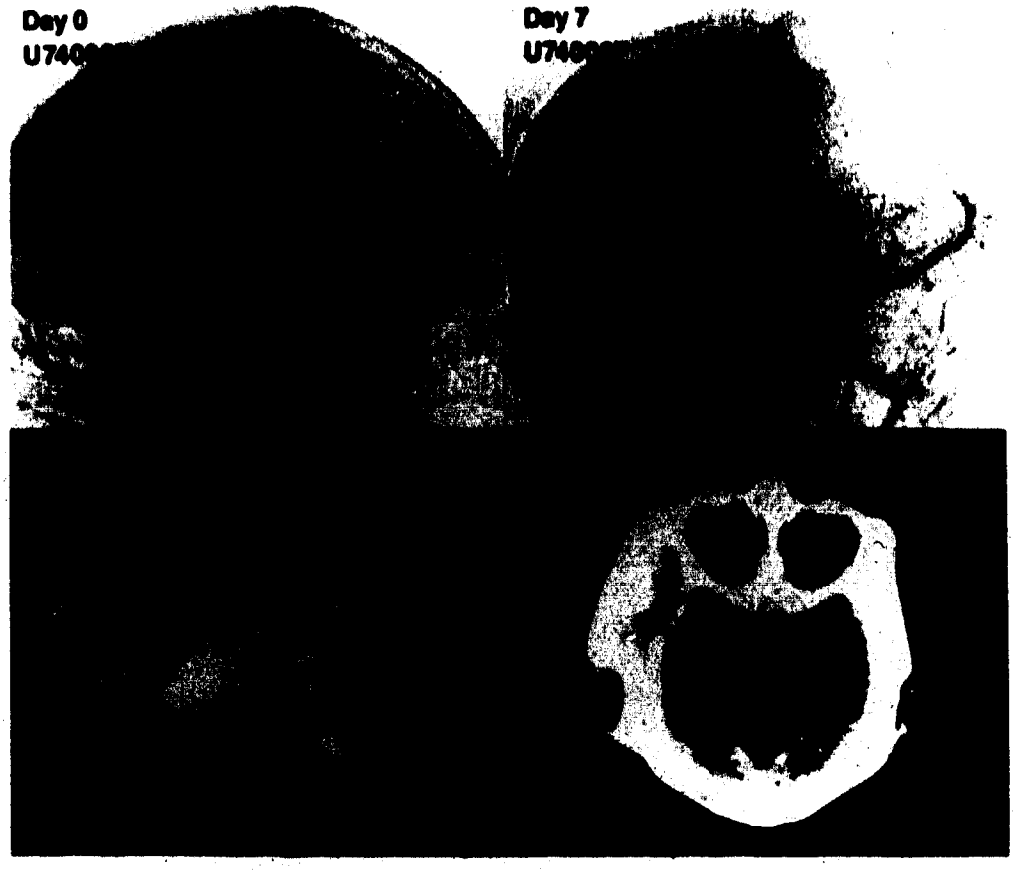


Figure 3

Top: Day 0 and Day 7 angiograms of Monkey 27, a U74006F treated animal, showing severe focal spasm of the proximal MCA by Day 7.
Bottom left: T·2 weighted MRI showing high intensity signal in right temporal-parietal area compatible with recent cerebral infarction.
Bottom right: Day 5 CT scan of same animal displaying cerebral infarction in the right temporal-parietal area.

Table II

Comparison of Treatments by Degree of Vasospasm in the Right Middle Cerebral Artery

Treatment	Degree of Vasospasm (%)* None	Mild	Moderate	Severe
U74006F n=20	6 (30)	3 (15)	7 (35)	4 (20)
Placebo n=20	0 (0)	2 (10)	8 (40)	10 (50)

*Change in vessel caliber
None = ± 10%
Mild = 11-30% decrease
Moderate = 31-50% decrease
Severe = > 50% decrease
p = 0.03

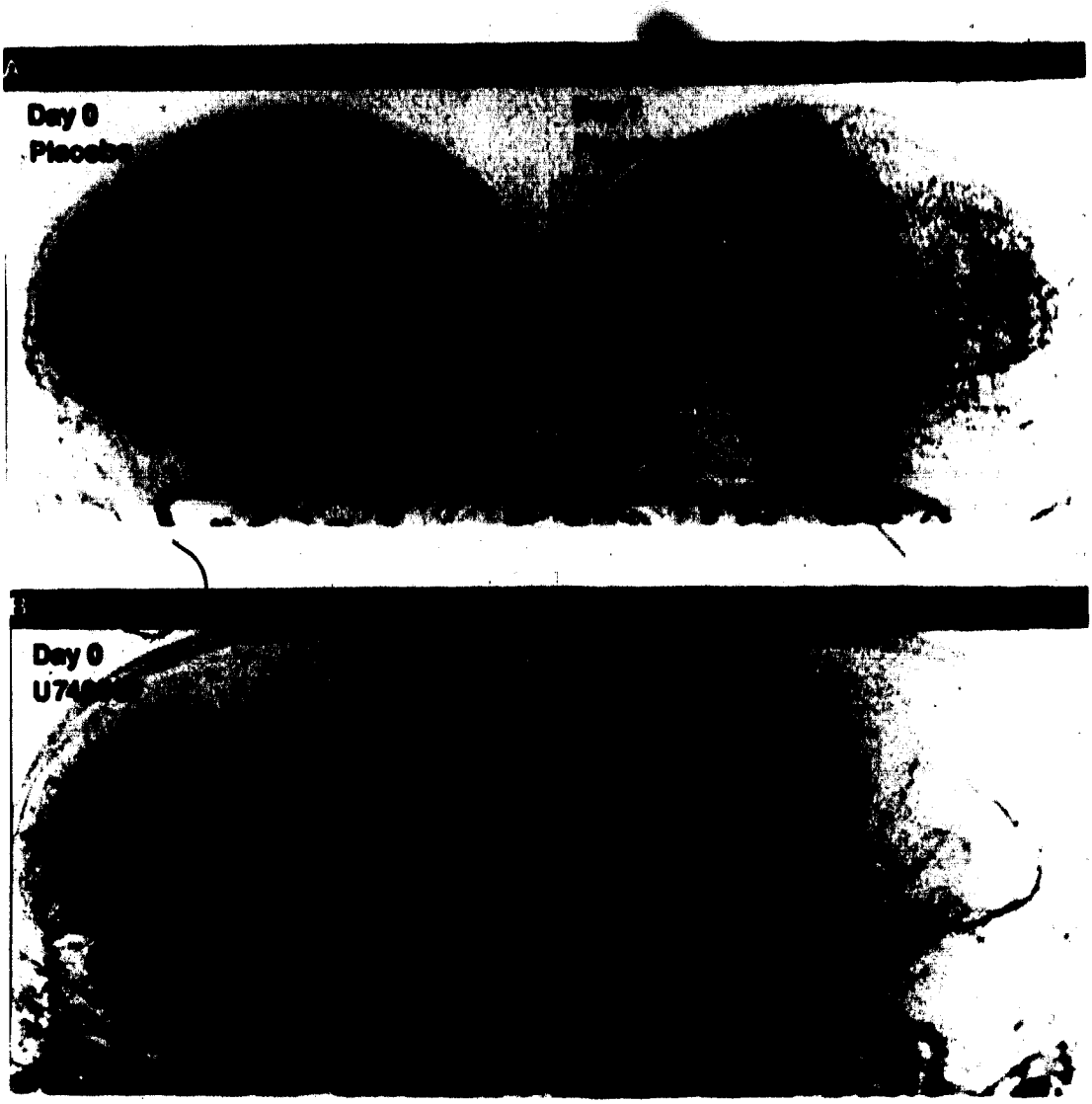


Figure 4

A: Day 0 and Day 7 angiograms of placebo animal showing moderate to severe spasm of the right-sided vessels.

B: Day 0 and Day 7 angiograms of a U74006F animal showing mild spasm of right-sided vessels.

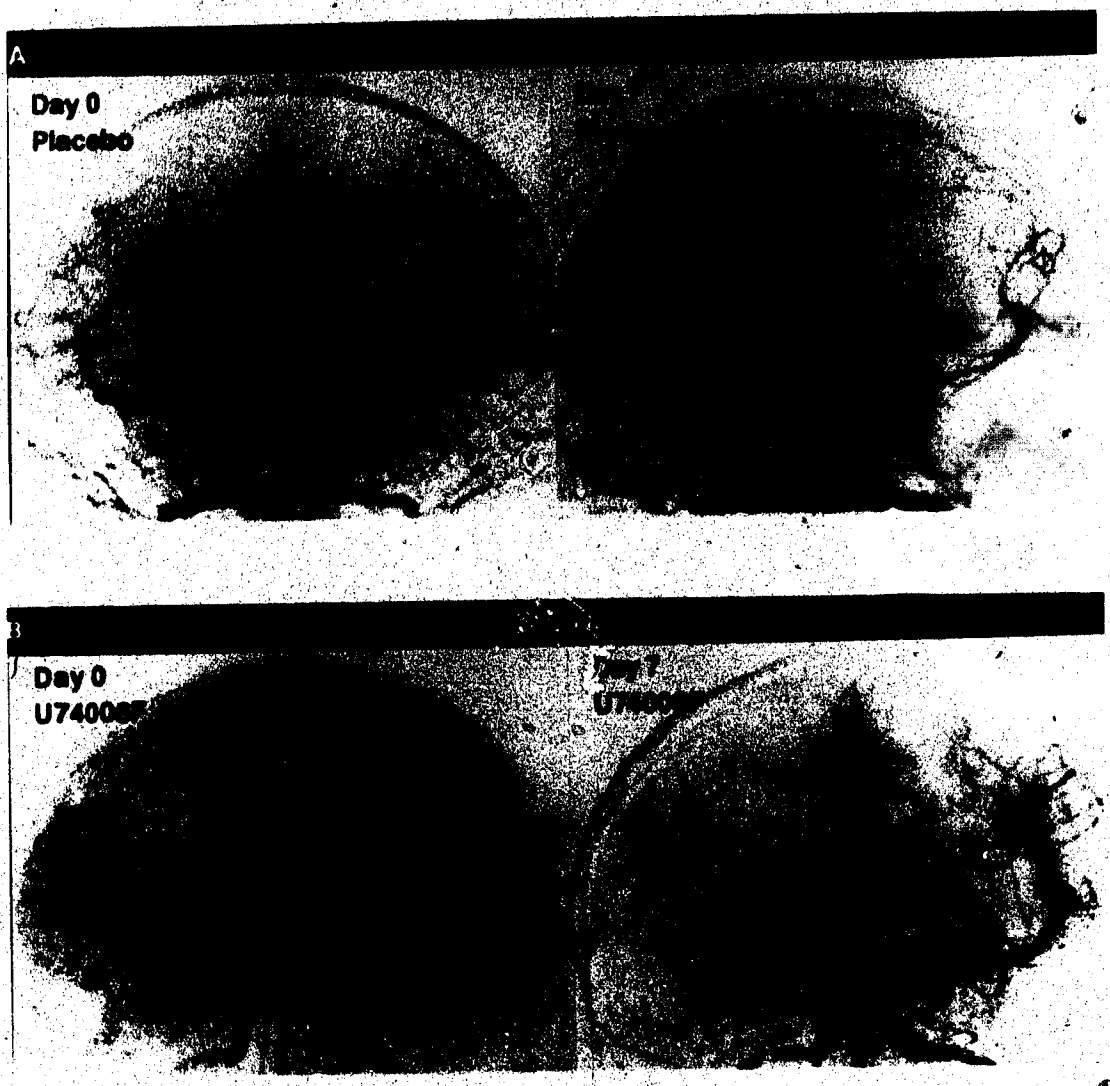


Figure 5

A: Day 0 and Day 7 angiograms of placebo animal showing moderate to severe spasm of the right-sided vessels.

B: Day 0 and Day 7 angiograms of a U74006F animal showing mild spasm of right-sided vessels.

D: Pathology

Pathological studies were carried out on 5 monkeys from each treatment group. All animals had remaining subarachnoid clot of comparable size. No evidence of epidural, subdural or intracerebral hematoma was found. Gross abnormalities were detected only in the 2 animals that developed DID's. The histological sections displaying these infarctions are seen in Figure 6. Note that the infarction in Monkey 17, the vehicle animal (A), was substantially larger than that in the U74006F treated animal (B). Both strokes were in the distribution of the right MCA. A gross pathological specimen displaying the size and distribution of subarachnoid clot is seen in Figure 7.

Electron microscopy (SEM and TEM) was used to examine the right and left C4-ICA's, A1-ACA's and MCA's. In the majority of animals, the left-sided vessels were normal. Monkey 17 showed changes of mild VSP in the left MCA and A1-ACA on both SEM and TEM. Representative scanning electron micrographs from Monkey 17 are displayed in Figure 8.

With SEM, the vessels from the clot side of both treatment groups were noted to be narrowed with a thickened arterial wall. Longitudinal endothelial folds accompanied this alteration and the extent of visible damage was in agreement with the degree of angiographic spasm (Figure 9). The vessels of several U74006F animals displayed changes of mild VSP only, while the majority of changes in the vehicle group were of moderate to severe degree (Figure 10). The SEM picture depended upon the site of arterial section and whether VSP was focal or diffuse. In both groups, the pathological changes appeared to be more pronounced in the vessel wall immediately adjacent to the perivascular clot.

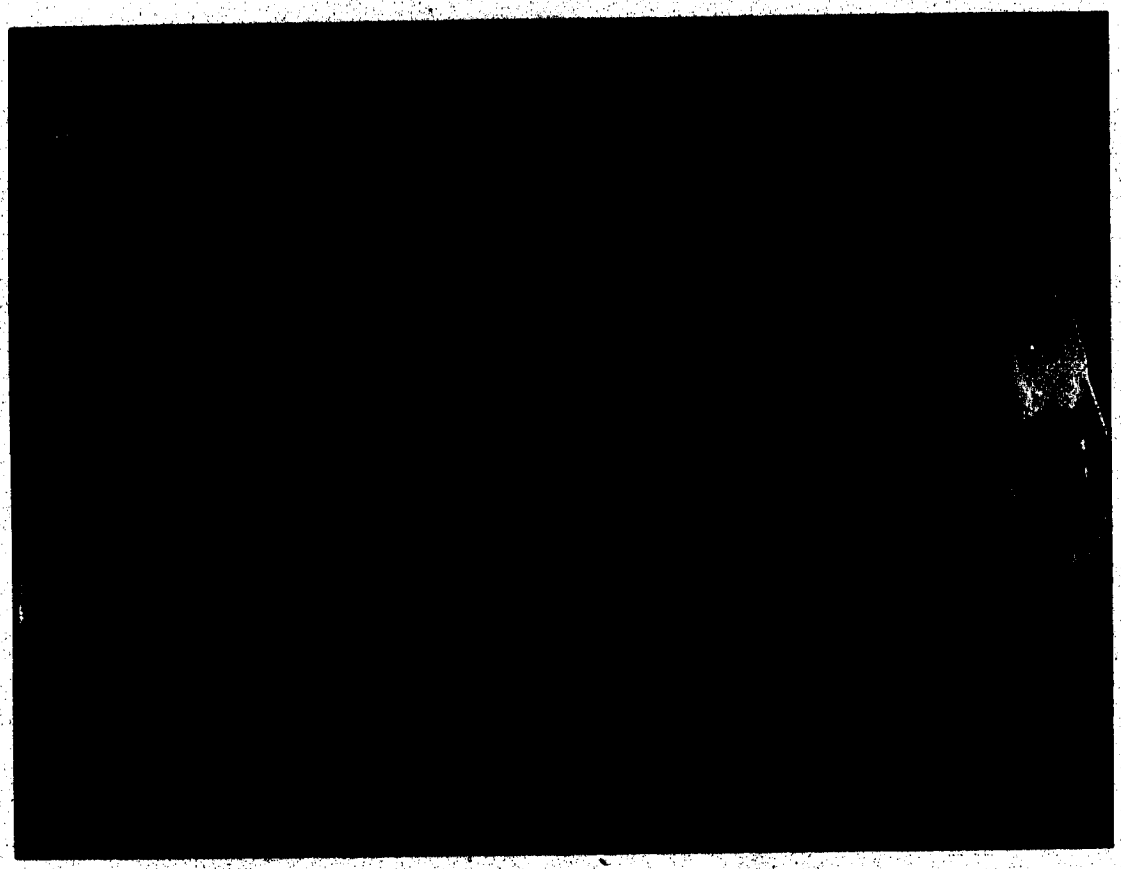


Figure 6

Brain sections from Monkey 17, a placebo animal, and Monkey 27, a U74006F treated animal. *denotes area of cerebral infarction.

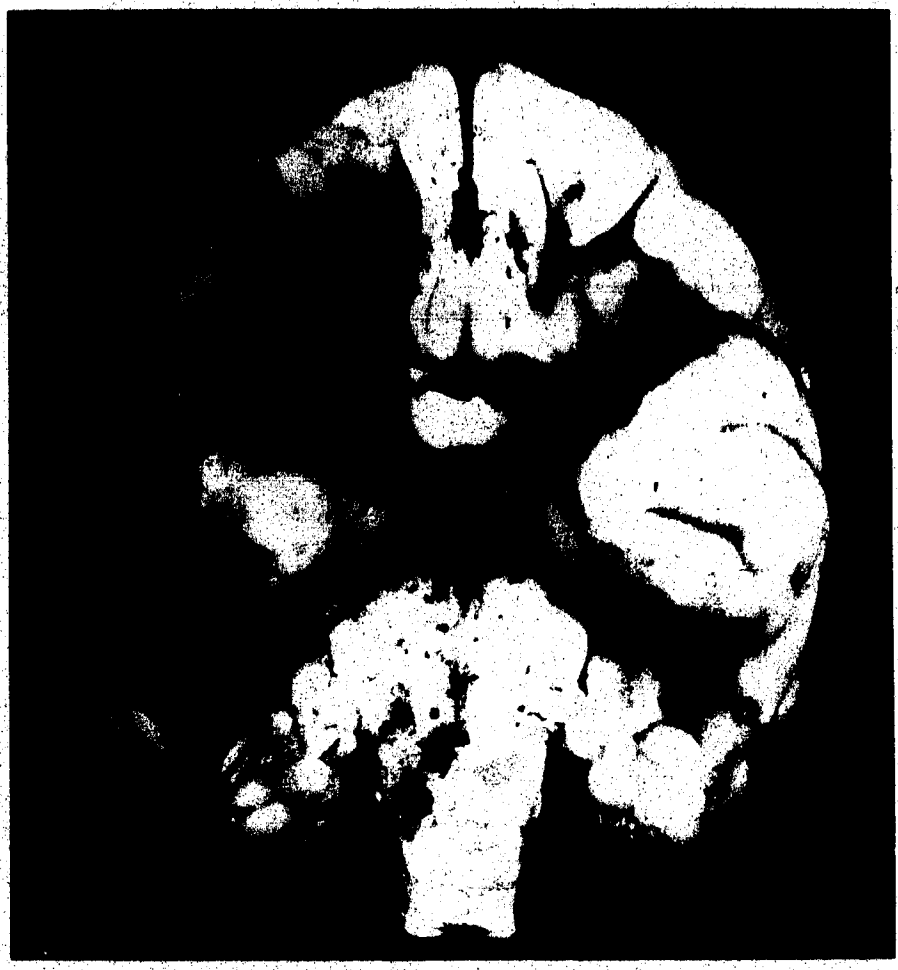


Figure . 7

Gross pathological specimen displaying the size and distribution of subarachnoid clot.

Figure 8

Scanning electron micrographs of vessels from Monkey 17. (bar=0.1 mm)
A: Right middle cerebral artery. Magnification 310x.
B: Close up view of the endothelial surface of the vessel in A. Magnification 1620x.
C: Left anterior cerebral artery showing changes of mild spasm. Magnification 200x.
D: Close up view of the endothelial surface of the vessel in C. Magnification 600x.

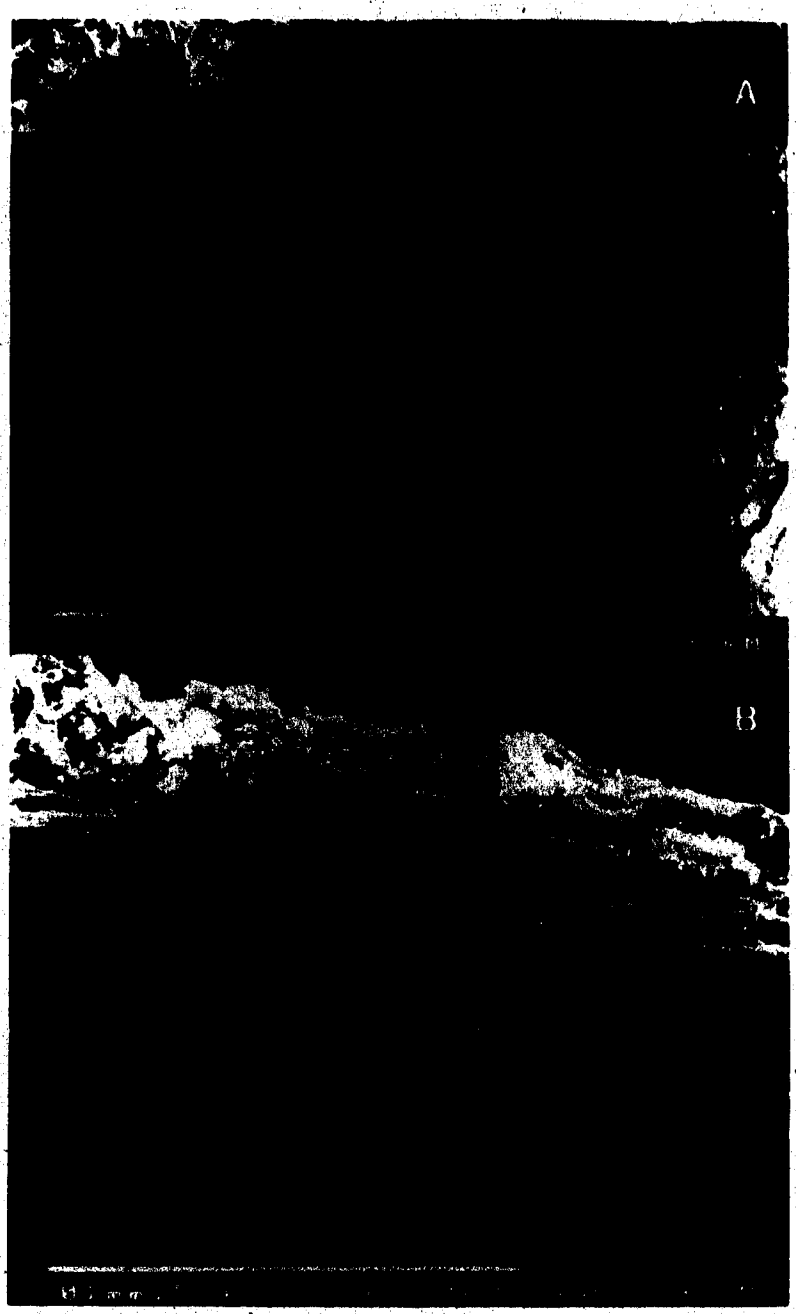


Figure 9

Scanning electron micrographs of the endothelial surface of clot side and non-clot side MCA's (bar=0.1 mm).
A: Clot side MCA. Magnification 2000x.
B: Non-clot side MCA. Magnification 1050x.

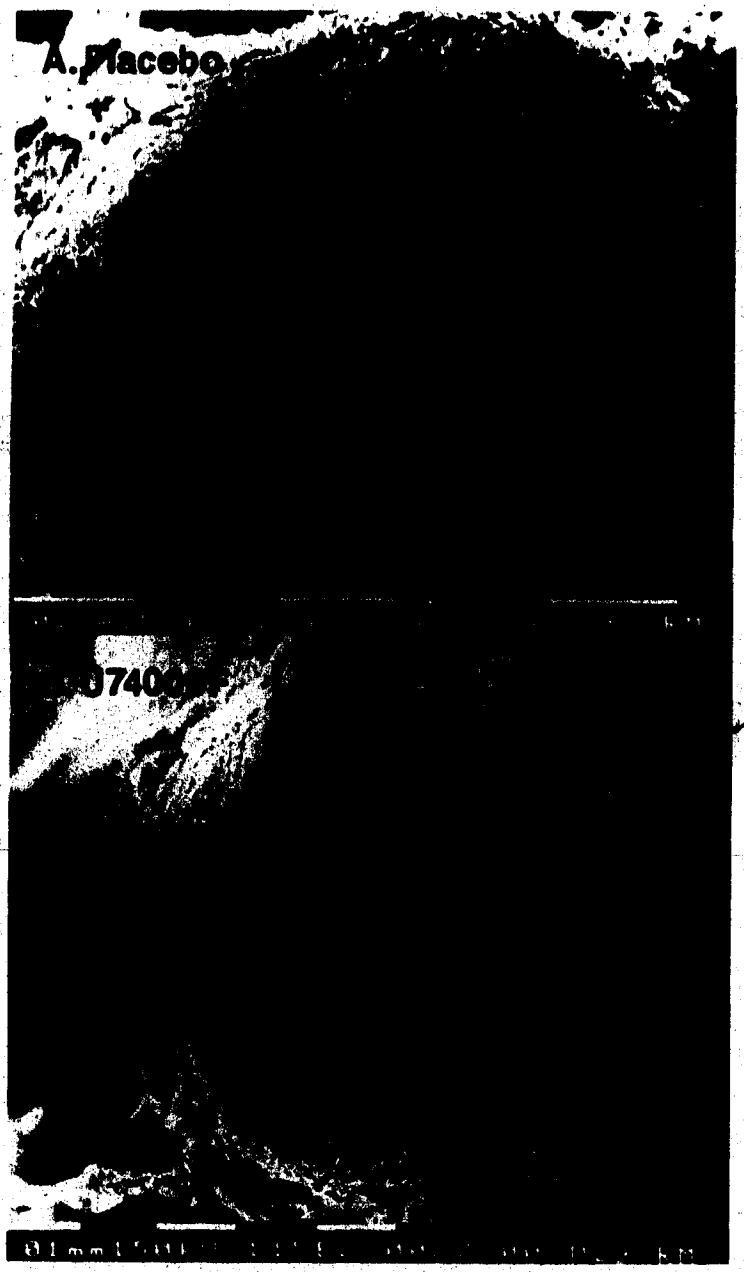


Figure 10

A: Scanning electron micrograph of the MCA from a placebo animal. Changes are consistent with severe VSP. Magnification 1200x (bar=0.1 mm).

B: Scanning electron micrograph of the MCA from a U74006F animal. Mild VSP is present. Magnification 725x (bar=0.1 mm).

On TEM, the spastic arteries demonstrated corrugation of the internal elastic lamina and intimal swelling. The degree of change again correlated with the severity of spasm. Both vehicle and U74006F animals showed extensive alterations with severe spasm (Figure 11), although as with SEM, changes in the U74006F group were generally milder (Figure 12) than those in vehicle animals. Myonecrosis was noted rarely.

Significant endothelial changes were detected in vessels with severe spasm. These changes consisted of alteration of endothelial cell shape, dissolution of intracellular organelles, and in some instances overt membrane damage (Figure 13). Breakdown of interendothelial junctions was seen occasionally although not a consistent finding (Figure 14).

E: HPLC Data

The GRADPROG equilibration curve is seen in Figure 15. The standard curves for AMP, ADP, ATP and PCr are displayed in Figures 16 to 19. Figure 20 shows the standard curve for protein analysis.

Results of the Day 7 cortical biopsies are given in Table 3. Although overall changes in phosphagen levels did not reach statistical significance, a reduction in ATP and phosphocreatine (PCr) occurred on the clot side in both treatment and control groups (Figure 21). The modified energy charge ratios (ATP/ADP+AMP and PCr/ADP+AMP) also decreased. Changes in the U74006F animals were less than 10% while a reduction of greater than 50% occurred in the placebo group. Changes in individual animals were categorized in the same manner as the angiographic results. Ischemia was defined as a reduction in the ATP/ADP+AMP ratio of greater than 10%. A decrease of 11-30% was said to be mild, 31-50% moderate, and greater than 50% severe. The degree

Figure 11

Transmission electron micrographs of middle cerebral arteries from placebo and U74006F treated animals. L denotes vessel lumen.

A: Clot side MCA of placebo animal. Magnification 5720x.
B: Non-clot side MCA of placebo animal. Magnification 4840x.
C: Clot side MCA of U74006F animal. Magnification 6480x.
D: Non-clot side MCA of U74006F animal. Magnification 3920x.

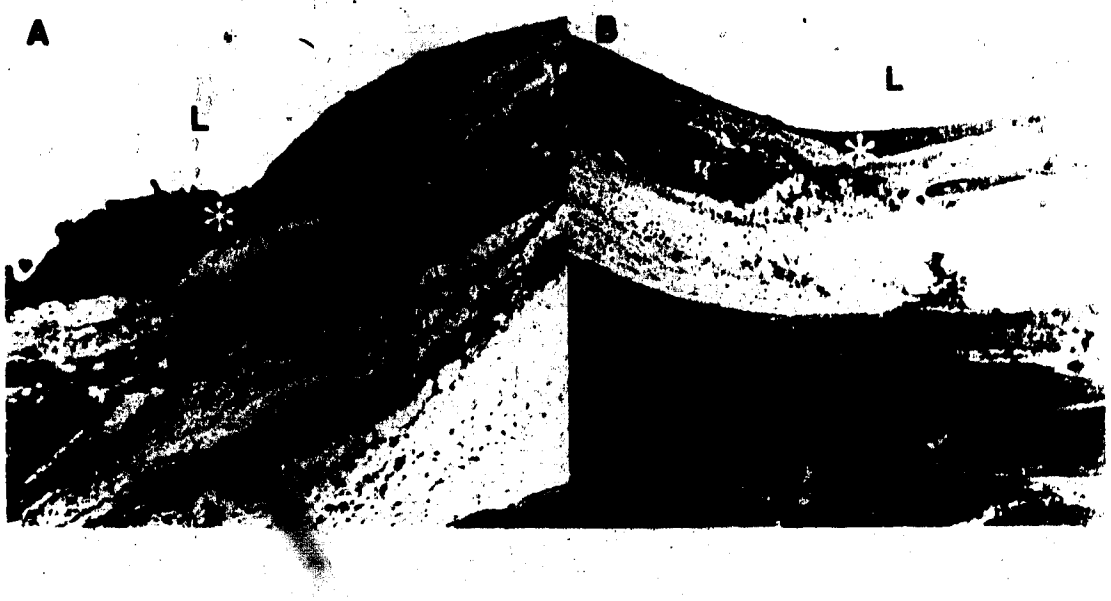


Figure 12

Transmission electron micrographs of the clot-side MCA of a U74006F treated animal. L denotes vessel lumen and * denotes endothelial layer.

A: High powered view showing mild endothelial alteration and intimal swelling. Magnification 12320x.

B: Low powered view of a different section of the same vessel as in A. Magnification 7260x.

Figure 13

Transmission electron micrographs of the middle cerebral artery of a placebo animal. L denotes vessel lumen.

A: High powered view of a single endothelial cell displaying evidence of membrane disruption. Magnification 94600x.

B: Lowered powered view of the same vessel as in A showing intimal swelling and endothelial damage. Magnification 9950x.

Figure 14

Transmission electron micrographs of the intimal surface of a middle cerebral artery from a placebo animal. L denotes vessel lumen.
A and B: Endothelial damage with early disruption of interendothelial junctions. Magnification A 9530x, B 13400x.
C: Overt disruption of interendothelial junction. Magnification 98700x.

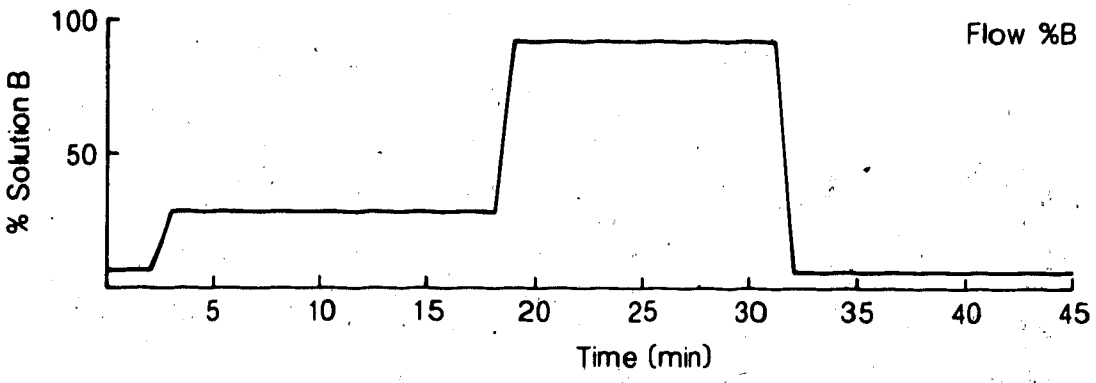


Figure 15

The Gradprog equilibration curve.

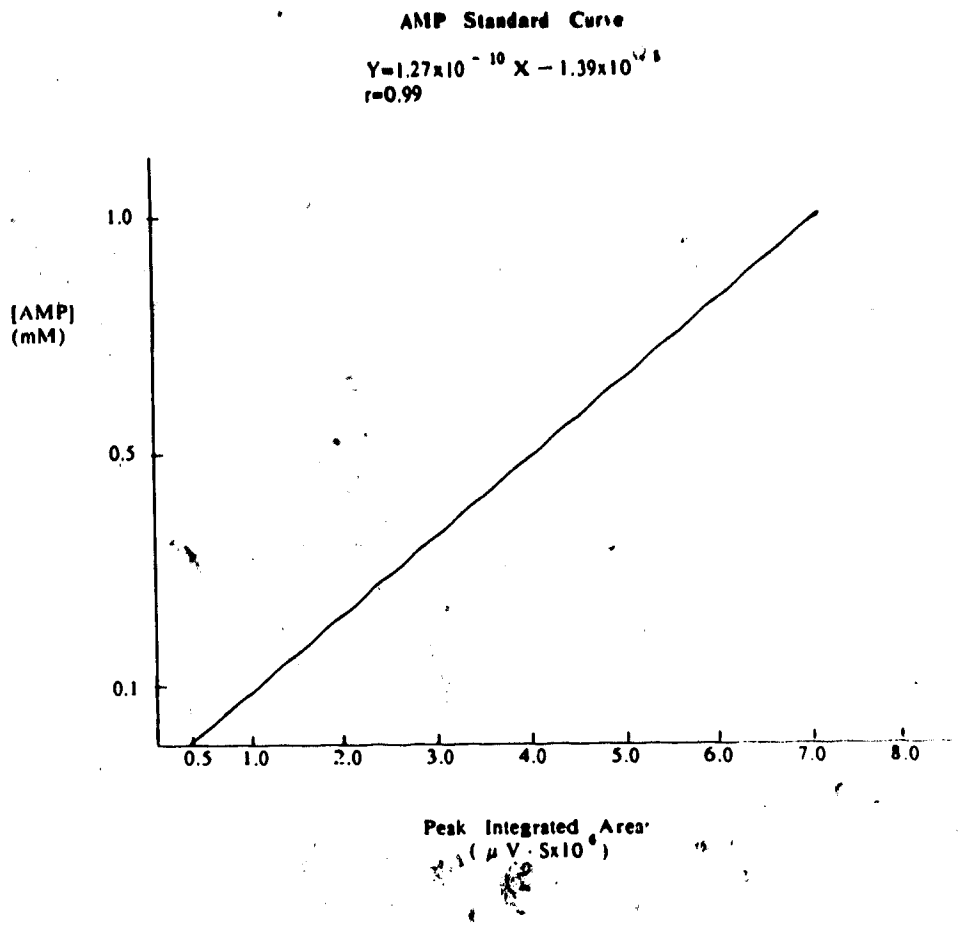


Figure 16

The adenosine monophosphate standard curve. * denotes standard curve equation, r: correlation coefficient.

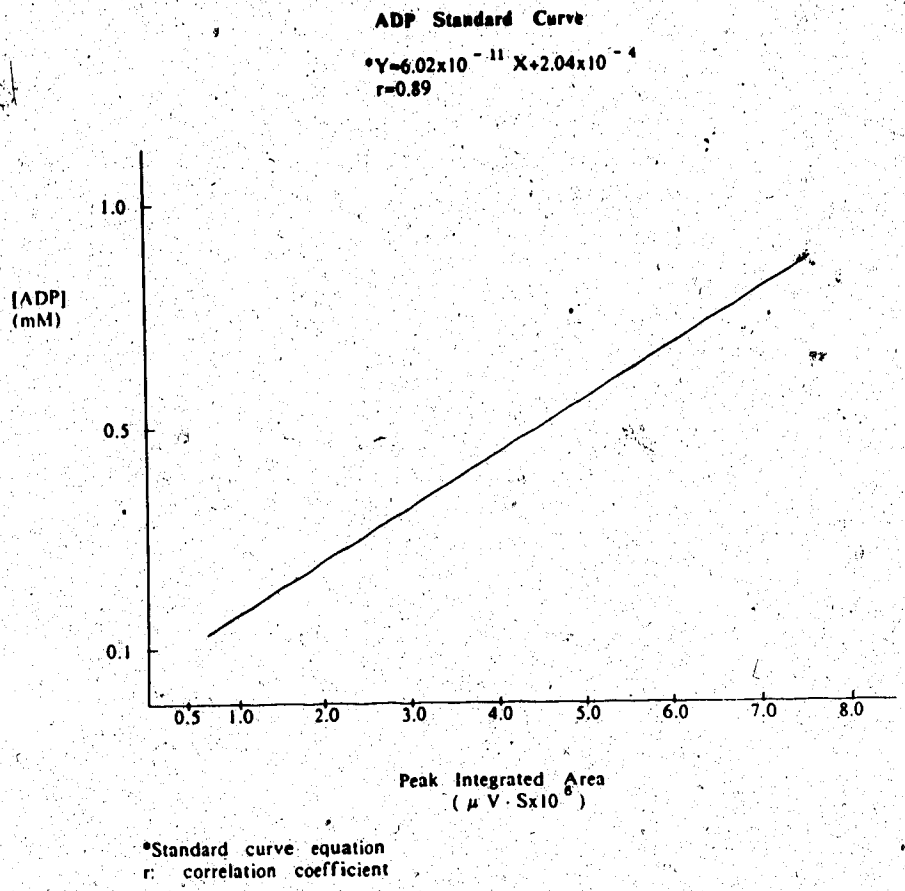


Figure 17

The adenosine diphosphate standard curve. * denotes standard curve equation, r: correlation coefficient.

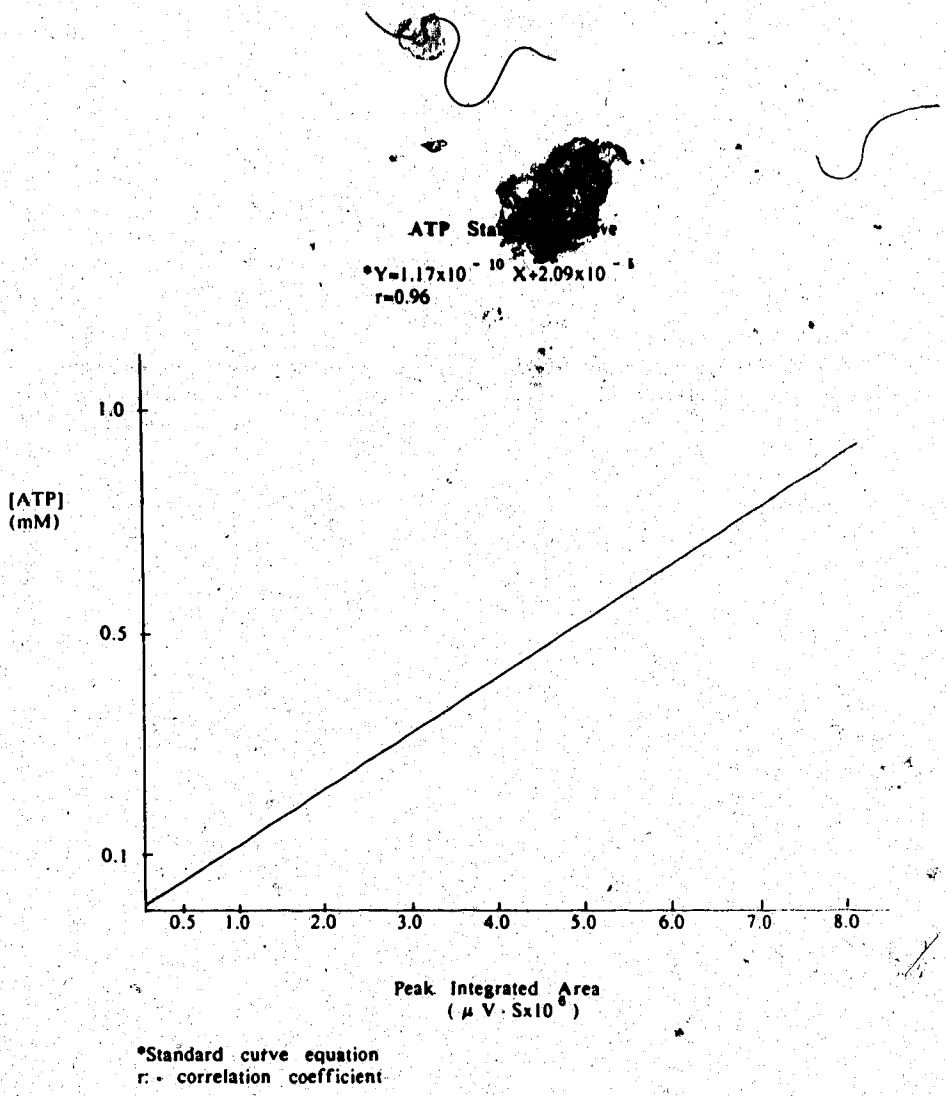


Figure 18

The adenosine triphosphate standard curve. * denotes standard curve equation, r: correlation coefficient.

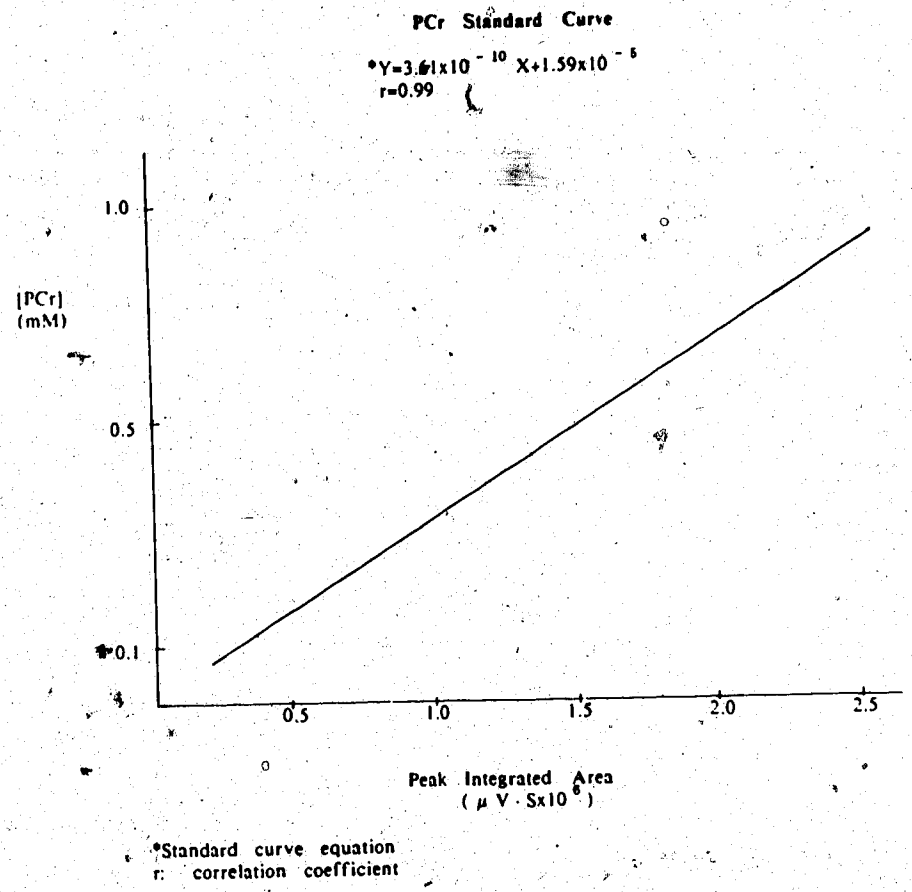


Figure 19

The phosphocreatine standard curve. * denotes standard curve equation, r: correlation coefficient.

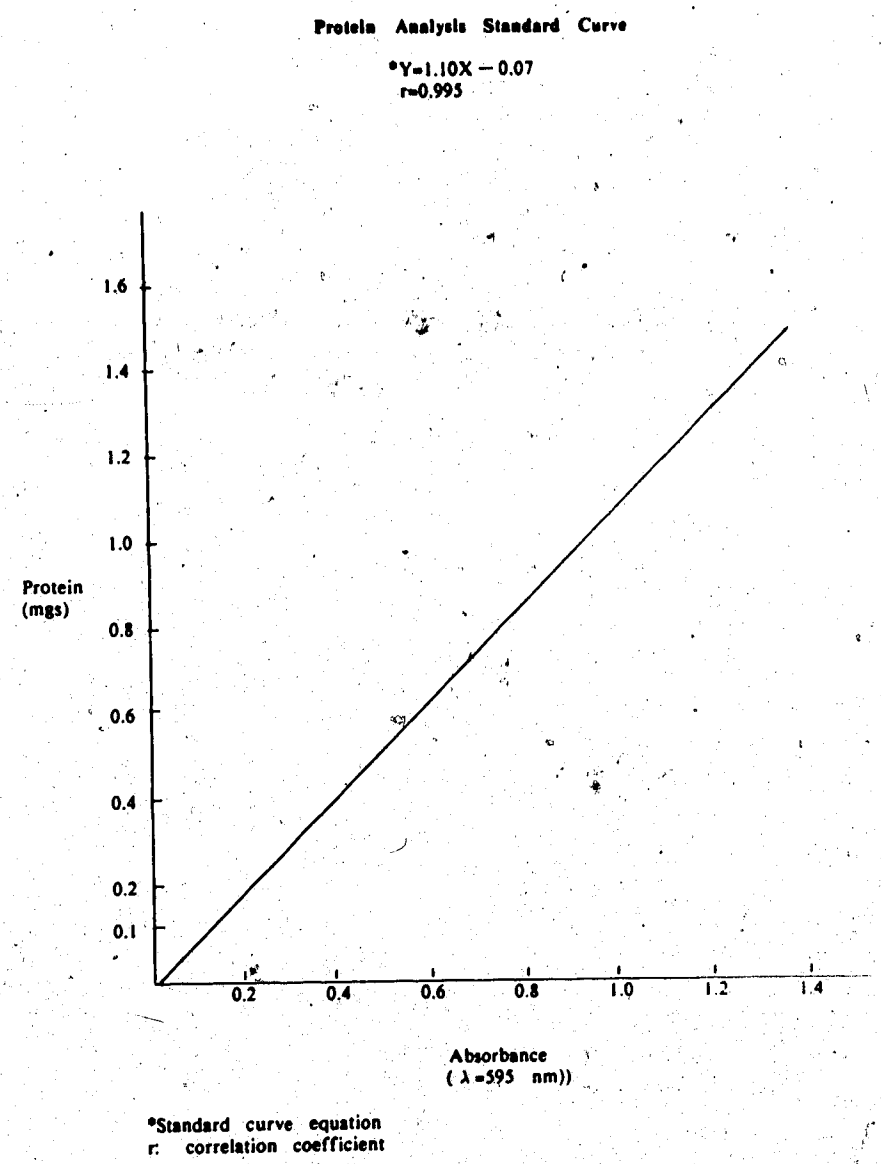


Figure 20

The protein analysis standard curve. * denotes standard curve equation, r: correlation coefficient.

Table III

A. Comparison of Treatments by Phosphagen Levels in Parietal Cortex

High Energy Phosphate μ gms/gm*	U74006F		Placebo	
	No Clot n=5	Clot n=5	No Clot n=5	Clot n=5
ATP	2.4 ± 0.8	1.8 ± 0.6	3.2 ± 0.7	1.6 ± 0.8
ADP	2.6 ± 0.3	1.9 ± 0.7	2.3 ± 0.5	2.4 ± 1.2
AMP	3.2 ± 1.7	2.5 ± 2.1	2.1 ± 1.1	2.4 ± 1.7
PCr	2.1 ± 0.7	1.6 ± 0.7	2.7 ± 0.7	1.1 ± 0.5
$\frac{ATP}{ADP+AMP}$	0.59 ± 0.4	0.55 ± 0.4	0.82 ± 0.3	0.38 ± 0.2
$\frac{PCr}{ADP+AMP}$	0.51 ± 0.4	0.49 ± 0.3	0.68 ± 0.3	0.27 ± 0.2

*All values are expressed as mean ± standard deviation
ATP = adenosine triphosphate
ADP = adenosine diphosphate
AMP = adenosine monophosphate
PCr = Phosphocreatine

B.

High Energy Phosphates*	U74006F n=5			Placebo n=5		
	NC	C	% ↓	NC	C	% ↓
$\frac{ATP}{AMP+ADP}$	0.59 ± 0.4	0.55 ± 0.4	7	0.82 ± 0.3	0.38 ± 0.2	54
$\frac{PCr}{AMP+ADP}$	0.51 ± 0.4	0.49 ± 0.3	4	0.68 ± 0.3	0.27 ± 0.2	60

*All values are expressed as mean ± standard deviation
NC: Nonclot or left side
C: Clot or right side
ATP: adenosine triphosphate
ADP: adenosine diphosphate
AMP: adenosine monophosphate

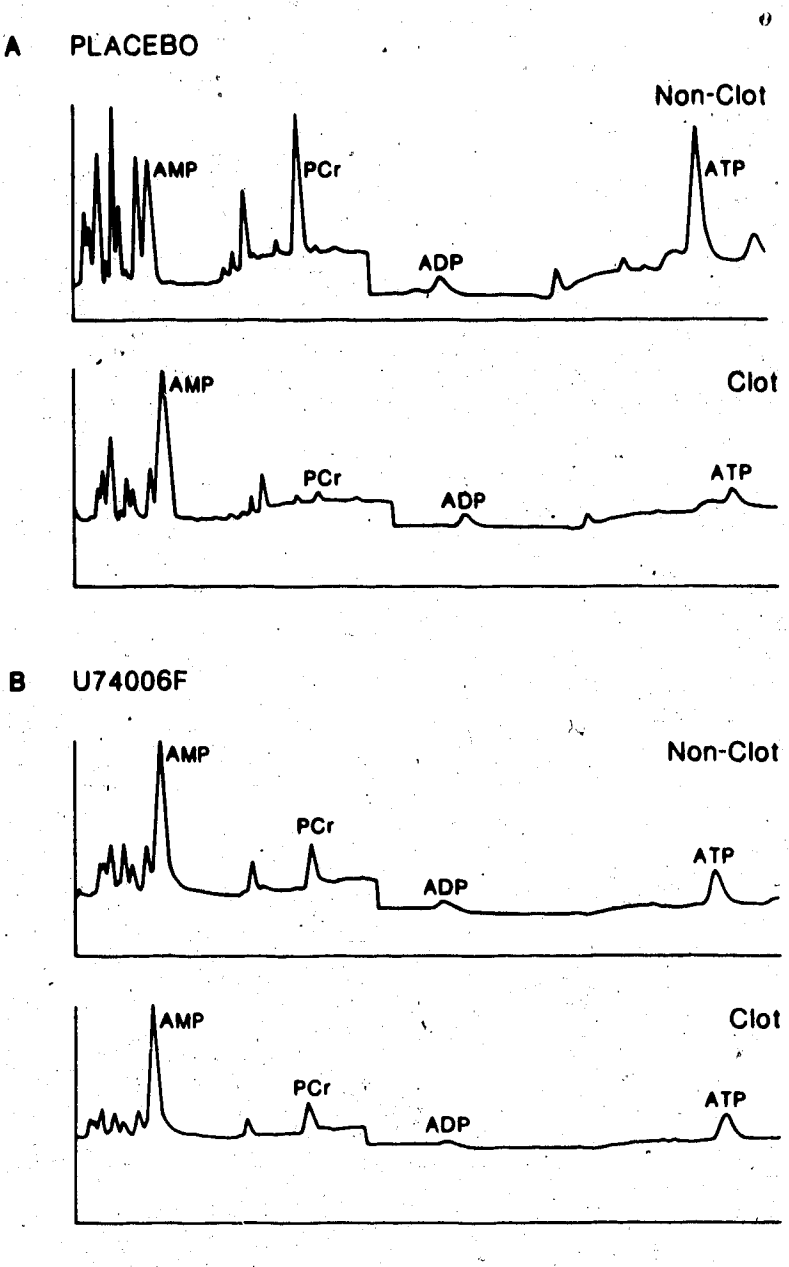


Figure 21

A: Representative HPLC tracing of a brain biopsy from a placebo animal showing a significant decrease in phosphocreatine (PCr) and adenosine triphosphate (ATP). The adenosine diphosphate (ADP) and adenosine monophosphate (AMP) peaks increased accordingly.

B: Tracing from a U74006F treated animal with no significant changes. Peaks are not as high as in A because of the smaller biopsy size.

of ischemia was moderate to severe in vehicle animals while absent to mild in the U74006F group (Table 4).

The degree of ischemia was correlated with the mean percentage VSP in the right MCA's of both treatment groups. It was found that the reduction in angiographic vessel caliber was less in the U74006F animals, and this may have accounted for the relative preservation of high energy phosphates in this group (Table 5).

In both animals that underwent HPLC analysis of their MCA's, a reduction in the ATP/ADP+AMP ratio occurred on the clot side relative to the non-clot side. One monkey was a control animal, the other received U74006F. In the control animal, the ratio was 50% less on the clot side compared to non-clot and this corresponded with a reduction in angiographic vessel caliber of 34%. The ratio was decreased by 75% in the U74006F animal, corresponding with a 49% reduction in angiographic vessel caliber. Phosphocreatine was measurable in arteries from the non-clot side of both animals but was undetectable in the spastic vessels.

F: Endothelium Dependent Relaxation

The technique used to remove cerebral vessels, prior to vessel relaxation studies, did not result in damage to the endothelial surface. Scanning electron micrographs of spastic and non-spastic vessels obtained in this manner, confirmed the presence of intact endothelium (Figure 22). The changes visible were in accordance with the degree of VSP (Figure 23).

The results of relaxation studies from Monkey 11, a U74006F animal, were not included in final analysis. Angiographically, the clot side

Table IV

Percent Decrease in ATP/AMP+ADP (Parietal Cortex)

Rx	Degree of Ischemia*			
	None	Mild	Moderate	Severe
U74006F n=5	2	3	0	0
Placebo n=5	0	0	3	2

*Change in ATP/ADP+AMP
None = ± 10%
Mild = 11-30% decrease
Moderate = 31-50% decrease
Severe = > 50% decrease

Table V

A. Correlation of Vasospasm with Change in ATP/ADP+AMP

R x	Mean % Vasospasm* of R MCA	Mean % Decrease in ATP/ADP+AMP**
U74006F n=5	36	7
Placebo n=5	52	54

*From animals in subgroup 2 only
**From parietal cortex biopsies
R MCA: right middle cerebral artery
ATP: adenosine triphosphate
ADP: adenosine diphosphate
AMP: adenosine monophosphate

B.

R x	Degree of Vasospasm*				Degree of Ischemia**			
	None	Mild	Mod	Sev	None	Mild	Mod	Sev
U74006F n=5	1	1	1	2	2	3	0	0
Placebo n=5	0	0	2	3	0	0	3	2

*Change in vessel caliber
**Change in ATP/ADP+AMP

None = 10%
Mild = 11-30% decrease
Mod = 31-50% decrease
Sev = > 50% decrease

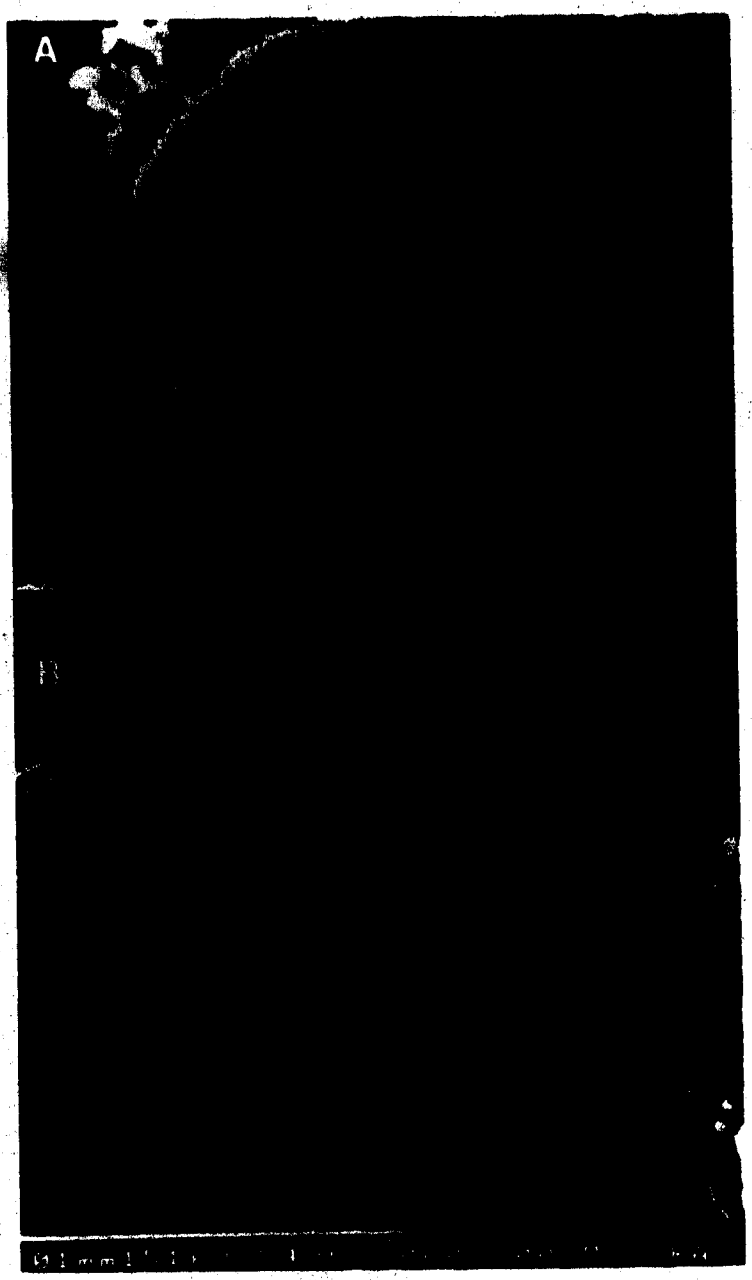


Figure 22

Scanning electron micrographs of the A1-ACA from a placebo monkey.
A: Low powered view showing changes consistent with mild-moderate VSP. Magnification 220x (bar=0.1 mm).
B: High powered view of the luminal surface depicting the intact endothelial layer. Magnification 880x (bar=0.1 mm).

Figure 23

Scanning electron micrographs showing endothelial changes of vessels in different degrees of spasm. (bar=0.1 mm)
A: Normal Vessel. Magnification 1620x.
B: Mild spasm. Magnification 2100x.
C: Moderate spasm. Magnification 1840x.
D: Severe spasm. Magnification 2830x.

vessels of this animal were in moderate VSP. The technique employed in removal of appropriate vessels was identical to that used in other animals. No damage to the vessels occurred at the time of removal. The samples were processed in the pharmacology lab in a manner indistinguishable from other vessels. The vessels from this animal were uniformly unreactive to the pharmacologic agents used. The reason for this is not entirely clear. It may be that the time taken between removal and relaxation studies was too long. It is possible that the Krebs bicarbonate solution had aged and was unable to sustain viability.

Dose response curves for BKN, ACH and ATP in placebo animals, can be seen in Figure 24. There was some difference between the ability of $PGF_{2\alpha}$ to produce vasoconstriction in clot vs non-clot side vessels. The former were significantly less reactive and the higher dose of $PGF_{2\alpha}$ was sometimes necessary to produce a response. Relaxations were expressed as a percentage of the $PGF_{2\alpha}$-induced contraction. Subarachnoid hemorrhage did not impair endothelium dependent relaxations due to BKN, nor the effects of ACH or ATP. When comparing the right MCA's of U74006F and vehicle treated animals, there was no significant difference in endothelium dependent relaxation (Figure 25).

The BASA, a vessel considered remote from the site of SAH in this model, reacted less to BKN, than the non-clot side MCA's of both treatment groups ($p < 0.05$). The responses to ACH and ATP were not significantly different.

The constriction generated by $PGF_{2\alpha}$ was reversed completely by high concentrations of papaverine on both the clot and non-clot sides, although the BASA proved to be marginally less reactive.

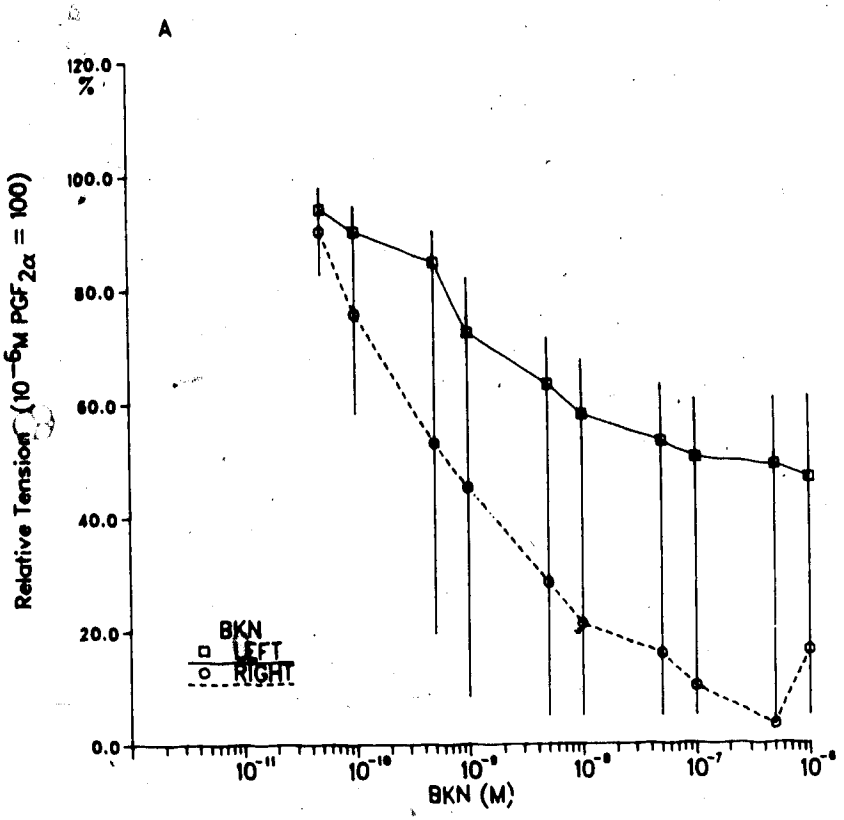


Figure 24A

Graphs showing relaxation values of right (clot side) and left (non-clot side) MCA's from placebo treated animals, in response to A. Bradykinin (BKN), B. Adenosine triphosphate (ATP) and C. Acetylcholine (ACH). Data are expressed as a percentage of the contraction induced by 10^{-6} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Vertical bars indicate standard error of the mean. (n=5 in each group).

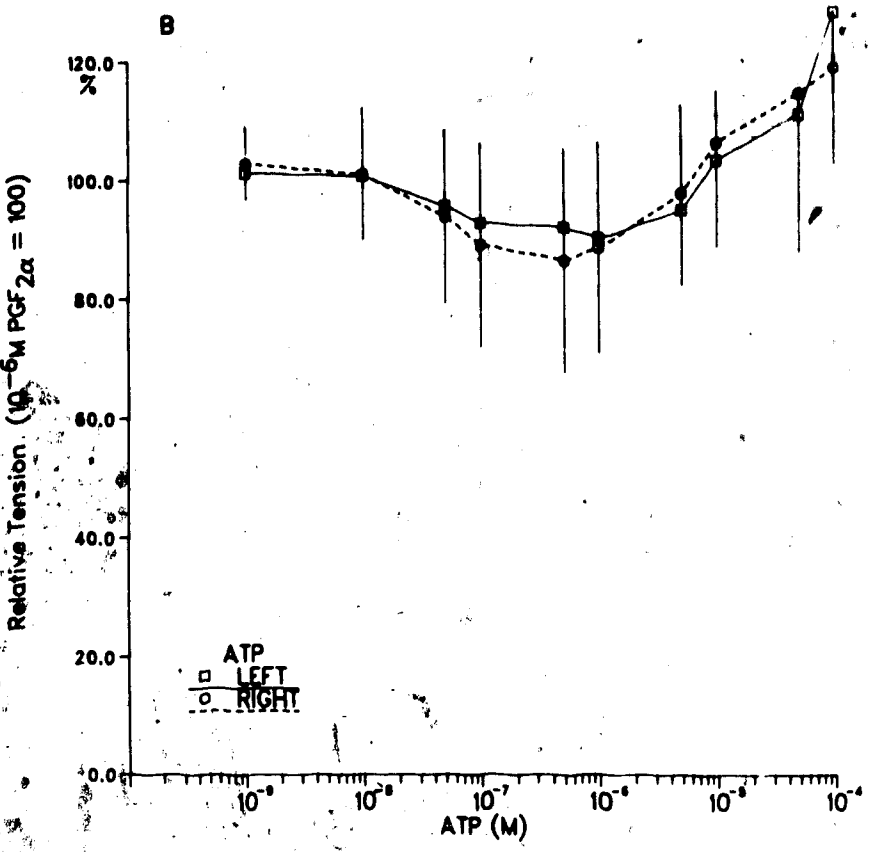

B
120.0
%
100.0
80.0
60.0
40.0
20.0
0.0
Relative Tension (10^{-6}M PGF$_{2\alpha}$ = 100)
ATP
LEFT
RIGHT
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
ATP (M)

Figure 24B

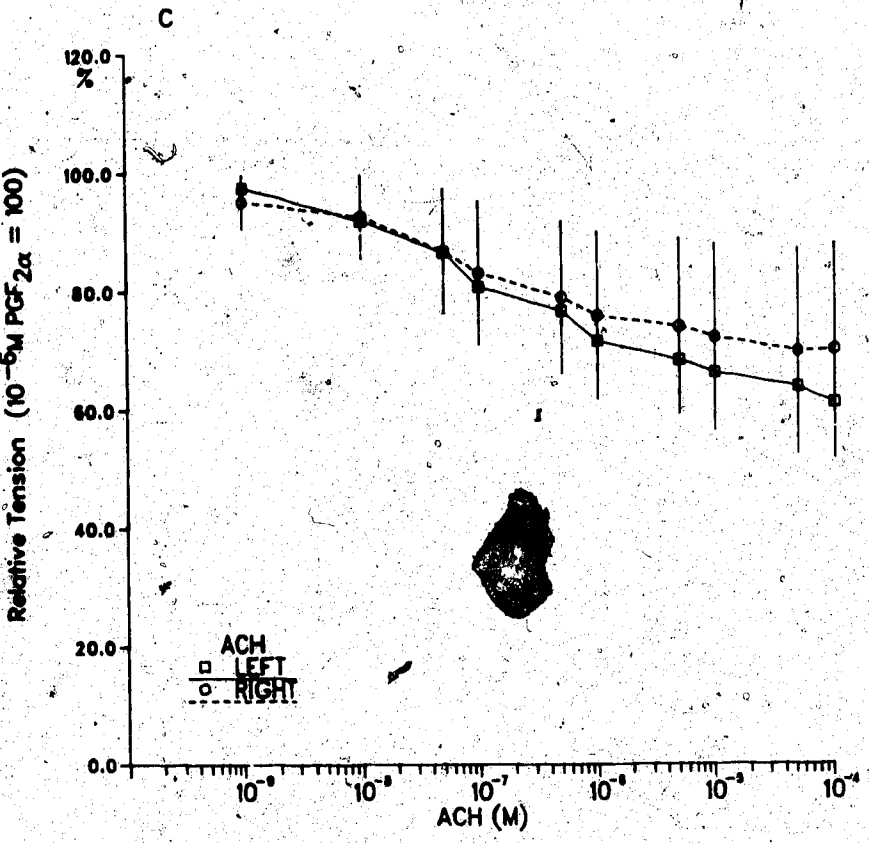

C
120.0
%
100.0
80.0
60.0
40.0
20.0
0.0
Relative Tension (10^{-6}M $PGF_{2\alpha}$ = 100)
ACH
LEFT
RIGHT
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
ACH (M)

Figure 24C

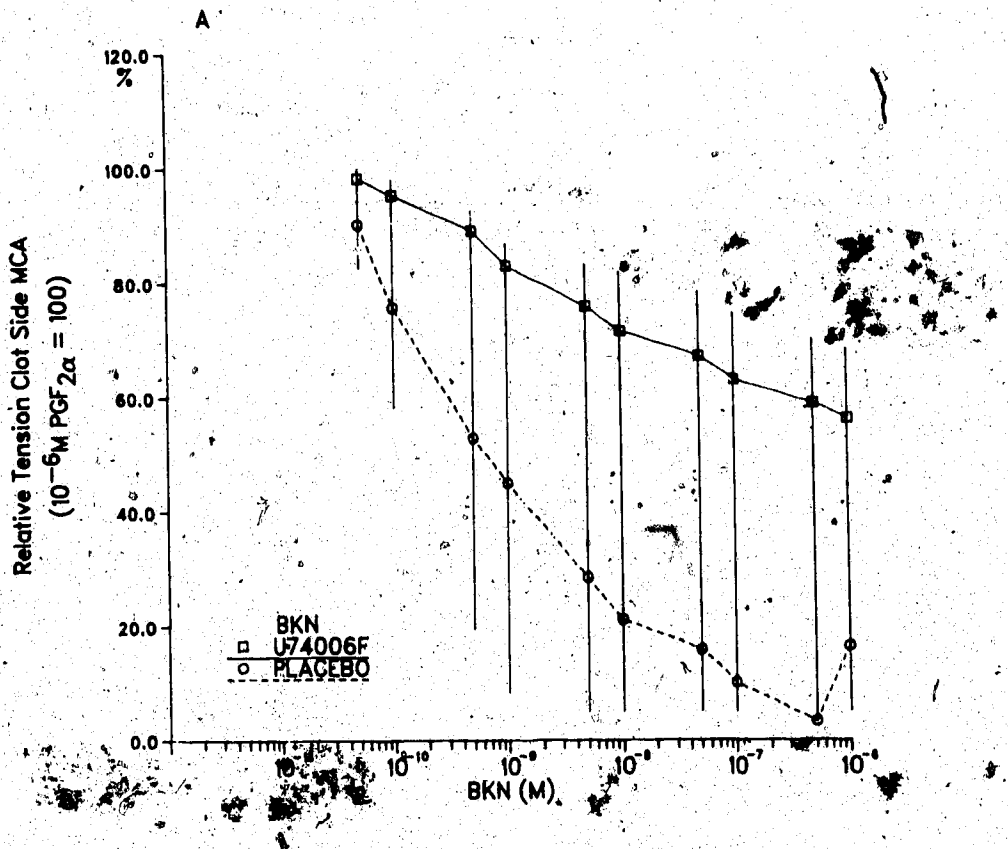


Figure 25A

Graphs showing relaxation values of clot-side MCA's from U74006F (n=4) and placebo treated (n=5) animals, in response to A. Bradykinin (BKN), B. Adenosine triphosphate (ATP) and C. Acetylcholine (ACH). Data are expressed as a percentage of the contraction induced by 10^{-6} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Vertical bars indicated standard error of the mean.

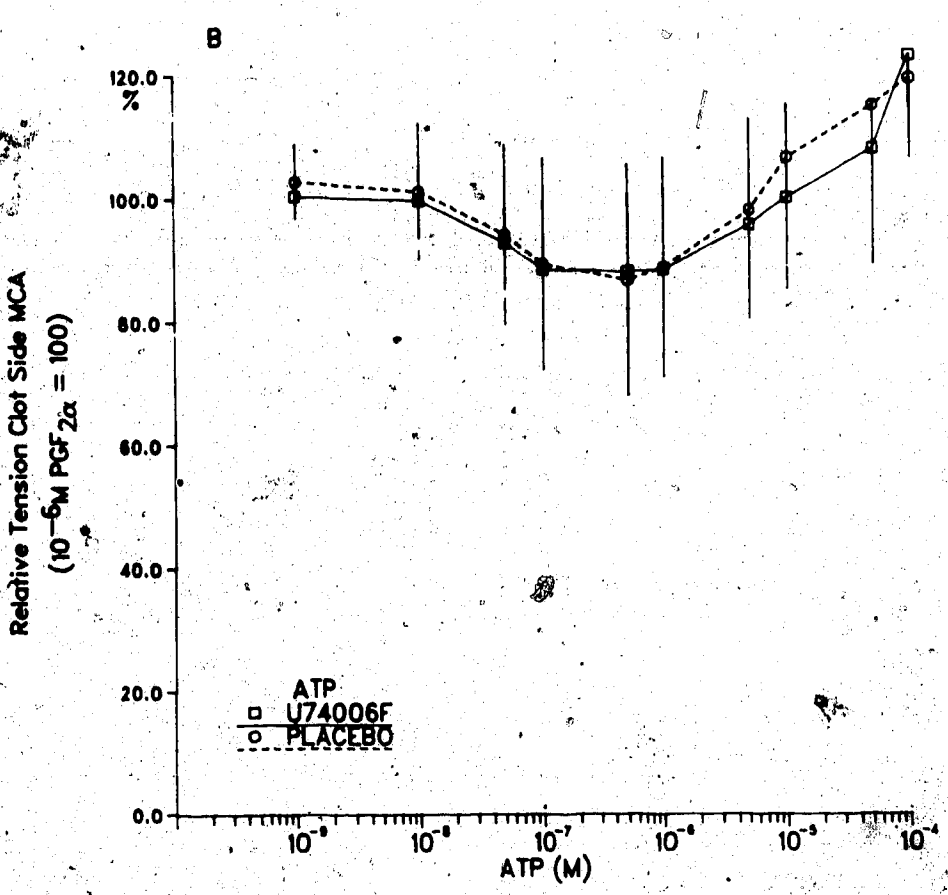

B
120.0
%
100.0
80.0
60.0
40.0
20.0
0.0
Relative Tension Clot Side MCA
(10^{-6}M $PGF_{2\alpha}$ = 100)
ATP
U74006F
PLACEBO
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
ATP (M)

Figure 25B

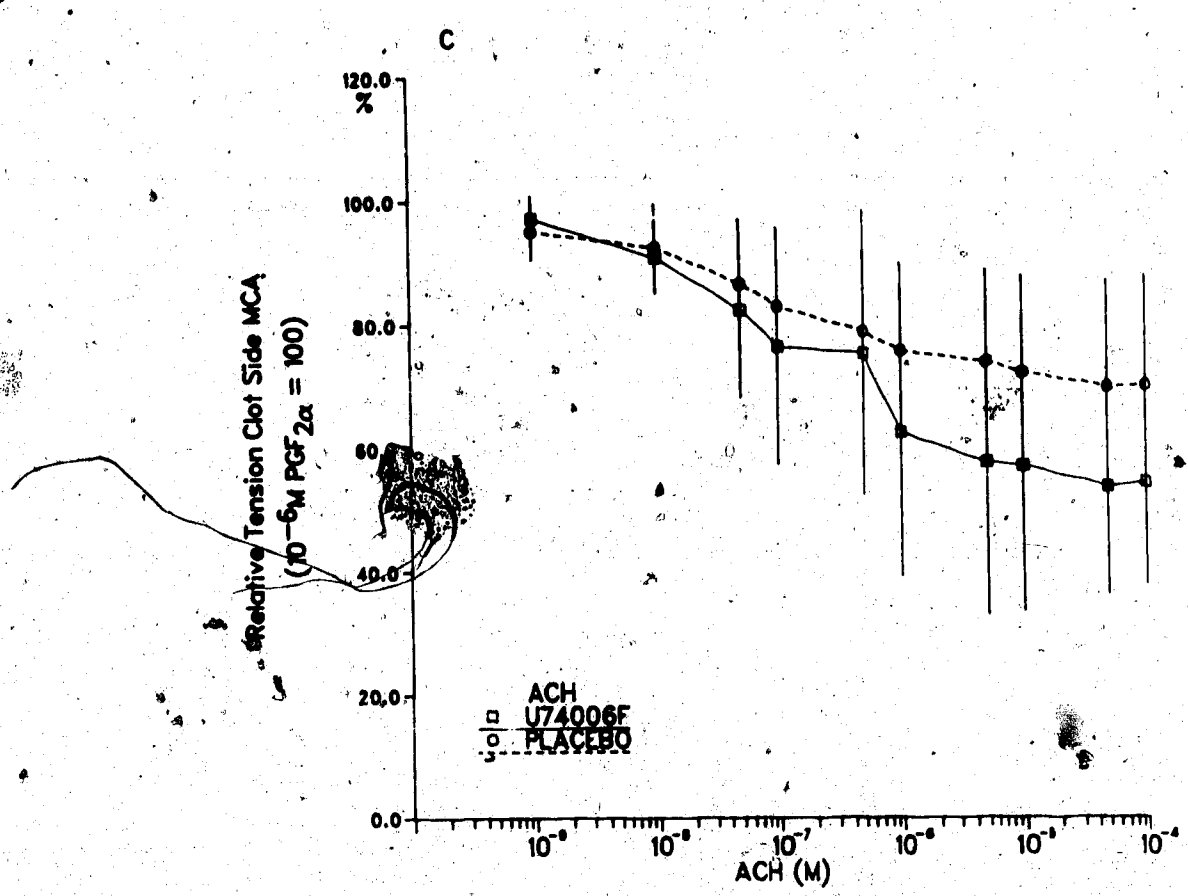

C
120.0
100.0
80.0
60
40.0
20.0
0.0
Relative Tension Clot Side MCA
(10^{-6}M PGF$_{2\alpha}$ = 100)
ACH
U74006F
PLACEBO
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-4}
ACH (M)

Figure 25C

CHAPTER FOUR: DISCUSSION

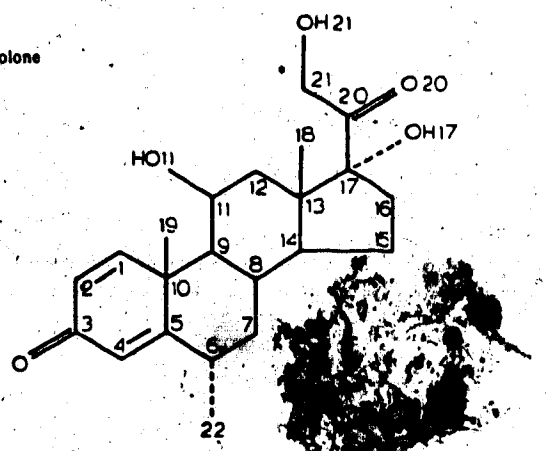
The present study demonstrated that U74006F inhibits the development of severe angiographic VSP following experimental SAH. Although significant VSP was present in both treatment groups, the arterial narrowing was more pronounced in vehicle treated animals. The beneficial effect on VSP was seen only in the clot side C3-ICA and MCA.

U74006F possesses no glucocorticoid or mineralocorticoid receptor binding activity and appears free of the side effects that accompany steroid usage.[18] Although the exact mechanism of action is uncertain, it resembles that of ∝-tocopheral. U74006F may scavenge superoxide anions and possibly acts as a membrane-localized iron chelator.[68] Recent work from the Upjohn labs has implicated another possible action. Using HPLC to measure levels of ∝-tocopheral, it was discovered that treatment with U74006F seemed to bolster endogenous vitamin E availability (Dr. Ed Hall, personal communication).

The 21-aminosteroid, U74006F (Figure 26) is at least 100 times more potent than methylprednisolone in inhibition of lipid peroxidation.[70] Its effectiveness on severe VSP corroborates the theory that free radicals and lipid peroxidation influence the development of VSP following aneurysmal SAH. The SEM results further support this hypothesis. The accentuation of arterial wall changes seen immediately adjacent to perivascular clot, suggests a vasotoxic etiology.

The mechanism by which free radicals contribute to the development of VSP, remains to be established. The chain reaction precipitated by free radicals may result in endothelial damage as seen in the arteries entombed by perivascular clot. Free radicals may contribute to the

A. Methylprednisolone



B. U74006F

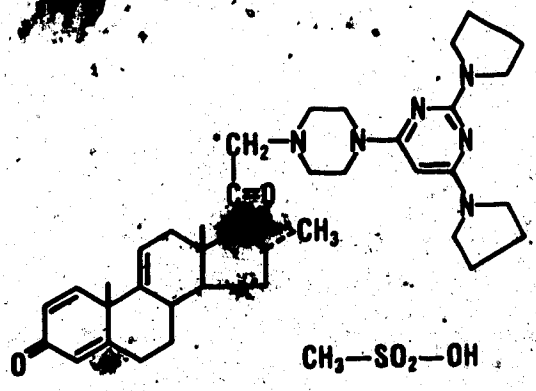


Figure 26

Comparison of the structure of Methylprednisolone with U74006F. denotes 21 carbon, the site of attachment of the amino group in U74006F.

alteration of vascular tone that accompanies SAH. In cell-free systems, hemoglobin impairs activation of soluble guanylate cyclase.[123] The increase in cGMP that normally accompanies the endothelium dependent relaxation produced by acetylcholine and the calcium ionophore A23187, is abolished by hemoglobin.[110] Other inhibitors of EDRF activity, generate superoxide anions and this is felt to be the basis for their biological effect.[120]

Aside from the vasotoxins liberated with red cell lysis, numerous vasoconstrictors or "spasmogens" have been implicated in the pathogenesis of VSP.[196] Free radicals can generate certain lipoxygenase metabolites non-enzymatically (enzyme-processing peroxidation) and several of these substances can be measured in the CSF of patients following SAH. These metabolites produce vasoconstriction and were found to correlate with the presence of VSP.[169]

The HPLC results suggested that U74006F may be a cerebroprotective agent and other free radical scavengers have been shown to attenuate cerebral ischemia and post-ischemic brain edema.[1,75] Our results did not reach statistical significance, but a definite tendency was evident. It is conceivable that the trend was a consequence of reduced VSP in U74006F animals, although again, differences between the two treatment groups were not statistically significant. Perhaps a larger sample size would have resolved this dilemma.

The HPLC technique employed in the present study has been developed recently. Similar HPLC analysis has been applied to other models of cerebral ischemia.[121] Frei et al have demonstrated that results of a cortical biopsy are not influenced by a previous biopsy from

the contralateral hemisphere.[47] In the majority of cases, published results are expressed in micromoles of phosphagen per gram of wet or dry weight. We elected to use grams of protein for the denominator because of its inherently greater accuracy.

The results from the MCA biopsies were interesting but inconclusive. It has been proposed that perivascular blood can obliterate the "rete vasorum" of the vessel wall impairing nutrition and oxygenation.[39,206] Several authors have speculated that vessel wall hypoxia may play a role in the pathogenesis of VSP.[125,183] Recent investigations have disclosed that endothelium dependent contractions may be involved in cerebral autoregulation.[94] Katusic and colleagues have demonstrated that anoxia contributes to the contraction of canine basilar arteries by affecting both endothelium and smooth muscle.[93] Anoxia may curtail the basal production of EDRF, and at the same time facilitate the release of endothelium-dependent constricting factor(s). In activated smooth muscle, anoxia is presumed to divert arachidonic acid from the cyclooxygenase pathway to the lipoxygenase pathway. Lipoxygenase products enhance the entry of calcium and contraction ensues.

Attempts have been made at determining the energy status of smooth muscle in cerebral and peripheral vessels.[11,78] Refinement is necessary before such techniques can yield reproducible results. With respect to cerebral VSP, further studies are required to establish cause and effect.

It has been suggested that free radicals and lipid peroxidation may impair endothelial function following SAH. Several papers have

reported the existence of spontaneously released EDRF.[63,111] With endothelial removal or damage, there is enhanced agonist-induced vasoconstriction. Abolition of spontaneously released EDRF has been implicated as the mechanism responsible.[127] Following SAH, the presence of hemoglobin, a selective inhibitor of EDRF, and endothelial damage should render the smooth muscle more susceptible to circulating and perivascular spasmogens.

Results of the pharmacological studies reported here were unable to support the idea that VSP arises from impairment of endothelium-dependent relaxation or indeed impairment of the action of endothelium-independent compounds. While the data with BKN showed considerable variability, there was no evidence of impaired relaxation to this agent in spastic vessels. Responses of both spastic and non-spastic arteries to AVH and ATP, which may have a minimal endothelial component, and to papaverine which is certainly a direct-acting smooth muscle relaxant, were likewise similar in all vessels examined. It is worth pointing out that the relaxations examined were those induced by $PGF_{2\alpha}$, and in the spastic vessels, this was in addition to constriction generated by the SVSP itself. Neither BKN nor papaverine were able to consistently produce relaxations below baseline in spastic vessels. These results imply that changes in spastic arteries preclude relaxation to the pharmacological agents tested.

Our findings are not in keeping with other studies. It is conceivable that the discrepancy relates to the different models used. Species variability in cerebral vasoreactivity is well known. Also, the majority of other reports deal with the effect of SAH on the BASA. We

were able to demonstrate that the BASA reacted to BKN differently than the non-clot side MCA, and both are equally remote from the site of SAH. Katusic and co-workers have shown that endothelium dependent contractions can be produced in canine basilar arteries using acetylcholine and the calcium ionophore A23187. These contractions are apparently due to cyclooxygenase products.[95] The pathologic importance of the heterogenous endothelium dependent behavior remains to be established.

Despite the difference in angiographic VSP between treatment groups, there was no disparity in endothelium-dependent relaxation. The variability of the responses may reflect a variety of sources of error, not the least of which is the variation in severity of spasm encountered in the clinical situation. A larger sample size may have helped to clarify the ambiguous results. Further study is required to resolve this perplexing issue. Endothelium-independent relaxation, as induced by papaverine, was also not affected by SAH. This result has been reported previously.[124]

The primate model of chronic cerebral VSP developed at the University of Alberta closely approximates the human situation. It produces angiographic VSP in over 90% of animals and the time course is identical to that seen clinically. Sham-operated animals are no longer included in current protocols as it has been demonstrated that craniotomy and arachnoid dissection alone does not give rise to VSP.[73,129,130] Approximately 5% of clot-animals develop a DID, despite the extensive collateral circulation seen in the cynomolgous macaque.

Over the last 2 to 3 decades, technological advancements have resulted in improved care and a better prognosis for most neurosurgical

patients. Despite this, and our better comprehension of CNS pathophysiology, a large number of patients continue to be devastated by SAH.

Vasospasm remains the principle antecedent of death and disability following aneurysmal SAH. Its prevention by manual or pharmacologic clot removal, seems to offer the best hope for the near future. However, not all patients are candidates for either thrombolysis or surgical clot evacuation. In this subgroup cytoprotective agents, like U74006F or calcium antagonists, may play a vital role in prevention of delayed ischemic deficits. The combination of a thrombolytic agent with cerebroprotection may prove optimal.

CHAPTER 5: RECOMMENDATIONS

Further study is required to define the role of U74006F in prophylaxis of chronic cerebral VSP and prevention of cerebral infarction.

Utilizing the primate model, different dosages should be investigated to explore the maximum potential of U74006F and possible side effects. Angiography and HPLC analysis should be undertaken in each case. Magnetic resonance spectroscopy of high energy phosphates is a reasonable non-invasive alternative to HPLC and may be less technically demanding. It is also available in numerous centers and therefore lends itself to comparisons between laboratory data and the clinical situation. It is conceivable that a lower dosage is as effective against severe VSP as the dosage used in the present study. Higher and lower dosages should be tried. The Upjohn Company has recently developed a HPLC technique for measuring drug concentrations in brain, which would permit further correlation of dosage with efficacy. Samples should be taken for analysis.

The cerebral ischemia results are inconclusive. It would be appropriate to examine a larger sample size in order to verify the trend demonstrated by the present study. Efforts should also be directed at obtaining vessel samples for HPLC analysis.

The department of pharmacology has developed a HPLC technique for measuring lipid peroxides in CSF. This technique could be applied to the primate model. It would augment our understanding of the pathophysiology of VSP, and assist in our search for the appropriate U74006F dosage.

The results of the EDRF study were disappointing. I believe the theory to be well founded and recent work from other laboratories supports it. A larger sample size may have permitted acquisition of conclusive results. If possible further study should be undertaken.

If further laboratory investigations substantiate the results of the present study, clinical trials should be forthcoming.

BIBLIOGRAPHY

1. Abe K, Yuki S, Kogure K: Strong attenuation of ischemic and postischemic brain edema in rats by a noval free radical scavenger. Stroke 19:480-485, 1988.
2. Alksne JF, Smith RW: Experimental models of spasm. Clin Neurosurg 24:216-227, 1977.
3. Alksne JF, Branson PF, Bailey N: Modification of experimental post subarachnoid vasculopathy with intracisternal plasmin. Neurosurgery 19:20-25, 1986.
4. Allen GS, Ahn HS, Preziosi TJ, et al: Cerebral arterial spasm - a controlled trial of nimodipine in patients with subarachnoid hemorrhage. NEJM 308:619-624, 1983.
5. Asano T, Tanishima T, Sasaki T, Sano K: Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid hemorrhage, in Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams and Wilkins, 1980, pp 190-201.
6. Asano T, Sasaki T, Koide T, Takakura K, Sano K: Experimental evaluation of the beneficial of an antioxidant on cerebral vasospasm. Neurol Res 6:49-53, 1984.
7. Asano T, Sasaki T, Koide T, Takakura K, Sano K: Role of the arachidonate cascade in the pathogenic mechanism of cerebral vasospasm following subarachnoid hemorrhage. Adv Prostaglandin Thromboxane Leukotriene Res 15:503-505, 1984.

8. Astrup J: Energy-requiring cell functions in the ischemic brain, their critical supply and possible inhibition in protective therapy. J Neurosurg 56:482-497, 1982.

9. Auer LM, Ito Z, Susuki A, Ohta H: Prevention of symptomatic vasospasm by topically applied nimodipine. Acta Neurochir 63:297-302, 1982.

10. Auer LM: Preventive nimodipine and acute aneurysm surgery. Heading for the control of complications after aneurysmal subarachnoid hemorrhage. Neurochir 28:87-92, 1985.

11. Barron JT, Glonek T, Messer JV: ^{31}P-nuclear magnetic resonance analysis of extracts of vascular smooth muscle. Atherosclerosis 59:57-62, 1986.

12. Beck DW, Adams HP, Flamm ES, Godersky JC, Loftus CM: Combination of aminocaproic acid and nicardipine in treatment of aneurysmal subarachnoid hemorrhage. Stroke 19:63-67, 1988.

13. Bell BA, Kendall BE, Symon L: Computed tomography in aneurysmal subarachnoid hemorrhage. J Neurol Neurosurg and Psychiatry 43:522-524, 1980.

14. Bevan JA: Mechanisms of contraction and relaxation of vascular smooth muscle. In: *Cerebral Vasospasm - 1987*, (in press).

15. Bolton TB: Mechanism of action of transmitters and other substances on smooth muscle. Physiol Rev 59:606-718, 1979.

16. Brandt L, Anderson KE, Edvinsson L, Ljunggren B: Effects of extracellular calcium and calcium antagonists on the contractile responses of isolated human pial and mesenteric arteries. J Cereb Blood Flow Metab 1:339-347, 1981.

17. Braughler JM, Pregenzer JF, Chase RL, Duncan LA, Jacobsen EJ, McCall JM: Novel 21-aminosteroids as potent inhibitors of iron-dependent lipid peroxidation. J Biol Chem 262:10438-10440, 1987.

18. Braughler JM, Chase RL, Neff GL, Yonkers PA, Day JS, Hall ED, Sethy VH, Lahti RA: A new 21-aminosteroid antioxidant lacking glucocorticoid activity stimulates adenocorticotropin secretion and blocks arachidonic acid release from mouse pituitary tumor (AtT-20) cells. J Pharmacol Exp Ther 244:423-427, 1988.

19. Bulkley GB: The role of oxygen free radicals in human disease processes. Surgery 94:407-411, 1983.

20. Butterfield JD, McGraw CP: Free radical pathology. Stroke 9:443-445, 1978.

21. Camp PE, Paxton HD, Buchan GC, Gahbauer H: Vasospasm after trans-sphenoidal hypophysectomy. Case report. Neurosurgery 7:382-386, 1980.

22. Casale AS, Bulkley GB, Bulkley BH, Flaherty JT, Gott VL, Gordon TJ: Oxygen free-radical scavengers protect the arrested globally ischemic heart upon reperfusion. Surg Forum 34:313-316, 1983.

23. Casteels R: Calcium ions and excitation contraction coupling in vascular smooth muscle cells, in Godfraind T, Herman AG, Willens D (eds): *Calcium Entry Blockers in Cardiovascular and Cerebral Dysfunction*. Boston: Martines Nijhoff, 1984, pp 45-52.

24. Chan PH, Yurko M, Fishman RA: Phospholipid degradation and cellular edema induced by free radicals in brain cortical slices. J Neurochem 38:525-531, 1982.

25. Cherry PD, Furchgott RF, Zawadzki JV, Jothianadan D: Role of endothelial cells in relaxation of isolated arteries by bradykinin. Proc Natl Acad Sci USA 79:2106-2110, 1982.

26. Chyatte D, Rusch N, Sundt T: Prevention of chronic experimental cerebral vasospasm with ibuprofen and high-dose methylprednisolone. J Neurosurg 59:925-932, 1983.

27. Chyatte D, Focle NC, Nichols DA, Sundt TM: Preliminary report: Effects of high dose methylprednisolone on delayed cerebral ischemia in patients of high risk for vasospasm after aneurysmal subarachnoid hemorrhage. 21:157-160, 1987.

28. Compton MR: The pathogenesis of cerebral infarction following the rupture of cerebral berry aneurysms. Brain 87:491-510, 1964.

29. Demopuolos HB, Flamm E, Seligman M, Pietronigro DD: Oxygen free radicals in central nervous system ischemia and trauma, in Autor AP (ed): *Pathology of Oxygen*. New York: Academic Press, 1982, pp. 127-155.

30. Drake CG: Management of cerebral aneurysm. Stroke 12:273-283, 1981.

31. Drake, CG: Formal discussion on Symon L: Vasospasm in aneurysm in Moosy J, Janeway R (eds): *Cerebrovascular Diseases. Seventh Conference*. New York: Grune and Stratton, pp 241-244.

32. Ebashi S,Mikawa T, Hirata M, Toyo-oka T, Nonomura Y: Regulatory proteins of smooth muscle, in Casteels R, Godfraind T, Ruegg JC (eds): *Excitation - Contraction Coupling in Smooth Muscle*. New York: Elsevier/North-Holland Biomedical Press, 1977, pp 325-334.

33. Ebashi S, Mikawa T., Hirata M, Ronomuray: The regulatory role of calcium in muscle. Ann NY Acad Sci 307:451-61, 1978.

34. Eisentrout C, Tomsick TA, Tew JM: Computed tomography findings as a prognostic factor in subarachnoid hemorrhage for ruptured cerebral aneurysms. Stroke 11:124, 1980 (Abstract).

35. Eldevik OP, Kristiansen K, Torvik A: Subarachnoid hemorrhage and cerebrovascular spasm. Morphological study of intracranial arteries based on animal experiments and human autopsies. J Neurosurg 55:869-876, 1981.

36. Espinosa F, Weir B, Boisvert D, Overton T, Castor W: Chronic cerebral vasospasm after large subarachnoid hemorrhage in monkeys. J Neurosurg 57:224-232, 1982.

37. Espinosa F, Weir B, Overton T, Castor W, Grace M, Boisvert D: A randomized placebo-controlled double-blind trial of Nimodipine after SAH in monkeys, Part 1: Clinical and radiological findings. J Neurosurg 60:1167-1175, 1984.

38. Espinosa F, Weir B, Overton T, Castor W, Grace M, Boisvert D: A randomized placebo-controlled double-blind trial of Nimodipine after SAH in monkeys, Part 2: Pathological findings. J Neurosurg 60:1176-1185, 1984.

39. Espinosa F, Weir B, Shnitka T: Electron microscopy of simian cerebral arteries after subarachnoid hemorrhage and after the injection of horseradish peroxidase. Neurosurgery 19:935-945, 1986.

40. Fein JM, Flor WJ, Cohan SL, et al: Sequential changes of vascular ultrastructure in experimental cerebral vasospasm.

Myonecrosis of subarachnoid arteries. J Neurosurg 41:49-58, 1974.

41. Ferrari R, Ceconi C, Curello S, Cargroni A, Medici D: Oxygen free radicals and reperfusion injury: the effect of ischemia and reperfusion on the cellular ability to neutralize oxygen toxicity. J Mol Cell Cardiol 18: (Suppl 4) 67-69, 1986.

42. Feurenstein G: Cardiac and vascular effects of leukotrienes. Adv Prostaglandin Thromboxane Leukotriene Res 16:299-308, 1986.

43. Findlay JM, Weir B, Steinke D, Tanabe T, Gordon P, Grace M: The effect of intrathecal thrombolytic therapy with tissue-type plasminogen activator on subarachnoid clot and chronic cerebral vasospasm in a primate model of subarachnoid hemorrhage. J Neurosurg (in press).

44. Fisher CM, Kistler JP, Davis JM: Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 6:1-9, 1980.

45. Flamm ES, Demopoulos HB, Seligman ML, Poser RG, Ransohoff J: Free radicals in cerebral ischemia. Stroke 94:445-447, 1978.

46. Flamm ES, Adams HP, Beck DW, Pinto RS, Marler JR, Walter MD, Godersky JC, Loftus CM, Biller J, Boarini DJ, O'Dell C, Banwart K, Kongable G: Dose-escalation study of intravenous nicardipine in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 68:393-400, 1988.

47. Frei HJ, Wallenfang Th, Poll W, Reulen HJ, Schubert R, Brock M: Regional cerebral blood flow and regional metabolism in cold induced oedema. Acta Neurochir 29:15-28, 1973.

48. Fridovich I: Superoxide radical: an endogenous toxicant. Ann Rev Toxicol 23:239-257, 1983.

49. Fujita K, Shingu T, Gi H, Araki O, Mutsunaga M, Handa H: Mechanism of cerebral vasospasm induced by oxyhemoglobin, in Brock M (ed): *Modern Neurosurgery*. Berlin: Springer-Verlag, 1982, pp 359-374.

50. Fujiwara S, Kuriyama H: Nicardipine actions on smooth muscle cells and neuromuscular transmission in the guinea-pig basilar artery. J Pharmacol Exp Ther 225:447-455, 1983.

51. Fujiwara S, Kassell NF, Sasaki T, Nakagomi T, Lehman RM: Selective hemoglobin inhibition of endothelium-dependent vasodilation of rabbit basilar artery. J Neurosurg 64: 445-452, 1986.

52. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in relaxation of arterial smooth muscle by acetylcholine. Nature 288:373-376, 1980.

53. Furchgott RF: Role of endothelium in responses of vascular smooth muscle. Circ Res 53:557-573, 1983.

54. Furchgott RF, Jothianandan D, Cherry PD: Endothelium-dependent responses: the last three years, in Vanhoutte PM, Vatner SF (eds): *Vasodilator Mechanisms*. Basel: Karger, 1984, pp 1-15.

55. Furchgott RF, Martin W, Cherry PD: Blockage of endothelium-dependent vasodilation by hemoglobin: a possible factor in vasospasm associated with hemorrhage. Adv Prostaglandin Thromboxane Leukotriene Res 15:499-502, 1985.

56. Furchgott RF: in Vanhoutte PM (ed): *Mechanisms of Vasodilatation Vol IV*. New York: Raven (in press).

57. Ganz P, Alexander RW: New insights into the cellular mechanisms of vasospasm. Am J Cardiol. 56:11E-15E, 1985.

58. Gardner TJ, Stewart JR, Casale AS, Downey JM, Chambers DE: Reduction of myocardial ischemic injury with oxygen-derived free radical scavengers. Surgery 94:423-432, 1983.

59. Germano I, Bartkowski HM, Cassel ME, Pitts LH: The therapeutic value of nimodipine in experimental focal ischemia, Neurological outcome and histopathological findings. J Neurosurg 67: 81-87, 1987.

60. Geyen W: Calcium and the contractile mechanism in heart and smooth muscle, in Opie LH (ed): *Calcium Antagonists and Cardiovascular Disease*. New York: Raven Press, 1984, Vol 9, pp 67-74.

61. Graham DI, MacPherson P, Pitts LH: Correlation between angiographic vasospasm, hematoma, and ischemic brain damage following SAH. J Neurosurg 59:223-230, 1983.

62. Granger DN, Rutili G, McCord J: Superoxide radicals in feline intestinal ischemia. Gastroenterology 81:22-29, 1981.

63. Griffith TM, Edwards DH, Lewis MJ, et al: The nature of endothelium-derived vascular relaxant factor. Nature 308:645-647, 1984.

64. Grogaard B, Parks DA, Granger DN, McCord JM, Forsberg JO: Effect of ischemia and oxygen radicals on mucosal albumin clearance in intestine. Am J Physiol 242: 9448-454, 1982.

65. Grotta JC, Spydell J, Pettigrew C, et al: The effect of nicardipine on neuronal function following ischemia. Stroke 17:213-219, 1986.

66. Grubb RL Jr, Raichle ME, Eichling JO, Gado MH: Effects of subarachnoid hemorrhage on cerebral blood flow, and oxygen utilization in humans. J Neurosurg 44:446-452, 1977.

67. Gurusinghe NT, Richardson AE: The value of [illegible]ed tomography in aneurysmal subarachnoid hemorrhage: a concept of the CT score. J Neurosurg 60:763-770, 1984.

68. Hall ED: Effects of 21-Aminosteroid U74006F on posttraumatic spinal cord ischemia in cats. J Neurosurg 68:462-465, 1988

69. Hall ED, Berry KP, Braughler JM: The 21-aminosteroid U74006F, a potent inhibitor of lipid peroxidation, protects against post-ischemic mortality and neuronal necrosis in gerbils. Stroke (in press).

70. Hall ED, Yonkers PA, McCall JM, Braughler JM: Effects of the 21-aminosteroid U74006F on experimental head injury in mice. J Neurosurg 68:456-461, 1988.

71. Halliwell B, Gutteridge JMC: Oxygen radicals and the nervous system. Trends Nerv Sys 8:22-26, 1985.

72. Handa J, Matsuda M, Nakasu Y, et al: Early operation of aneurysmal subarachnoid hemorrhage - use of nicardipine, a calcium channel blocker. Nippon Geka Hokan 53:619-630, 1984.

73. Handa Y, Weir BKA, Nosko M, Mosewich R, Tsuji T, Grace M: The effect of timing of clot removal on chronic vasospasm in a primate model. J Neurosurg 67:558-564, 1987.

74. Harper AM, Craigen L, Kazda S: Effect of the calcium antagonist Nimodipine on cerebral blood flow and metabolism in the primate. J Cereb Blood Flow Metab 1:349-356, 1981.

75. Harris RJ, Lindquist C, Kamiya K, Kuyama H, Branston NM, Symon L: The role of leucotrienes in the formation of cerebral edema. J Cereb Blood Flow Metab 3:s285-s286, 1983.

76. Hartshorne DJ: The contractile apparatus of smooth muscle and its regulation by calcium, in Cross MF, Barnes CD (eds): *Vascular Smooth Muscle: Metabolic, Ionic, and Contractile Mechanisms*. New York: Academic Press, 1982, pp 135-161.

77. Hawk JR, Ausman JI, Erickson, DL, Maxwell RE: Vasospasm following transcranial removal of large pituitary adenomas. Report of three cases. J Neurosurg 50:229-232, 1979.

78. Hellstrand P, Vogel HJ: Phosphagens and intracellular pH in intact rabbit smooth muscle studied by ^{31}P-NMR. Am J Physiol 248:C320-C329, 1985.

79. Heros RC, Zervas NT, Varsos V: Cerebral vasospasm. Surg Neurol 5:354-362, 1976.

80. Heros RC, Zervas NT, Varsos V: Cerebral vasospasm after subarachnoid hemorrhage: an update. Ann Neurol 14:599-608, 1983.

81. Hoover RL, Robinson JM, Karnovsky MJ: Adhesion of polymorphonuclear leukocytes to endothelium enhances the efficacy of detoxification of oxygen-free radicals. Am J Path 126:258-268, 1987.

82. Imaizumi S, Kayama T, Suzuki J: Chemiluminescence in hypoxic brain - the first report: correlation between energy metabolism and free radical reaction. Stroke 15:1061-1065, 1984.

83. Ishige N, Pitts LH, Pogliani L, Hashimoto T, Nishimura MC, Bartkowski HM, James TL: Effects of hypoxia on traumatic brain injury in rats: Part 2, changes in high energy phosphate metabolism. Neurosurgery 20:854-858, 1987.

84. Johnson R, Potter JF, Reid RG: Arterial spasm and subarachnoid hemorrhage: mechanical considerations. J Neurol Neurosurg Psychiatry 21: 68, 1958.

85. Kajikawa H, Ohta T, Yoshikawa Y, et al: Cerebral vasospasm and hemoglobins - clinical and experimental studies. Neurol Med Chir 19:61-71, 1979.

86. Kamiyama K, Okada H, Suzuki J: The relation between cerebral vasospasms and superoxide - application of an experimental model of vasospasms induced by oxyhemoglobin in cats. Neurol Med Chir 21:201-209, 1981.

87. Kanamaru K, Waga S, Kojima T, Fujimoto K, Itoh H: Endothelium-dependent relaxation of canine basilar arteries. Part I: difference between acetylcholine and A23187-induced relaxation and involvement of lipoxygenase metabolite(s). Stroke 18:932-937, 1987.

88. Kanamaru K, Waga S, Kojima T, Fujimoto K, Niwa S: Endothelium-dependent relaxation of canine basilar arteries, part 2: inhibition by hemoglobin and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage. Stroke 18:938-943, 1987.

89. Kassell NF, Peerless SJ, Durward QJ, Beck DW, Drake CG, Adams HP: Treatment of ischemia deficits from vasospasm with intravascular volume expansion and induced arterial hypertension. Neurosurgery 11:337-343, 1982.

90. Kassell NF, Sasaki T, Colohan ART, Nazar G: Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke 16:562-572, 1985.

91. Kassell NF, Torner JC: Epidemiology of intracranial aneurysms. Int Ancos Clin 20:13-17, 1982.

92. Kassell NF: The natural history and treatment outcome of SAH: comments derived from the national cooperative aneurysm study, in Battye R (ed): *Calcium Antagonists: Possible Therapeutic Use in Neurosurgery*. New York: Raven Health Care Communications, 1983, pp 24-29.

93. Katusic ZS, Vanhoutte PM: Anoxic contractions in isolated canine cerebral arteries. J Cardiovasc Pharmacol 8 (Suppl 8): S97-S101, 1986.

94. Katusic ZS, Shepherd JT, Vanhoutte PM: Endothelium dependent contraction to stretch in canine basilar arteries. Am J Physiol 252:H671-H673, 1987.

95. Katusic ZS, Shepherd JT, Vanhoutte PM: Endothelium-dependent contractions to calcium ionophore A23187, Arachnidonic acid and acetylcholine in canine basilar arteries. Stroke 19:476-479, 1988.

96. Kawakami Y, Shimamura Y: Cisternal drainage after early operation of ruptured intracranial aneurysm. Neurosurgery 20:8-14, 1987.

97. Kim P, Yaksh TL, Romero SD, Sundt TM: Production of uric ac in cerebrospinal fluid after subarachnoid hemorrhage in dogs: investigation of the possible role of xanthine oxidase in chronic vasospasm. Neurosurgery 21:39-44, 1987.

98. Kirber MT, Singer JJ, Walsh JV Jr.: Stretch-activated channels in freshly dissociated smooth muscle cells. Biophys J 51:252a, 1987.

99. Kistler JP, Crowell RM, Davis KR, Heros R, Ojemann RG, Zerva T, Fisher CM: The relation of cerebral vasospasm to the extent and location of subarachnoid blood visualized by CT scan: A prospective study. Neurology 33:424-426, 1983.

100. Kiwak KJ, Moskowitz MA, Levine L: Leukotriene production in gerbil brain after ischemic insult subarachnoid hemorrhage, and concussive injury. J Neurosurg 62:865-869, 1985.

101. Knuckey NW, Fox RA, Surveyor I, Stokes BAR: Early cerebral blood flow in computerized tomography in predicting ischemia after cerebral aneurysm rupture. J Neurosurg 62:850-855, 1985.

102. Kontos HA, Wei EP, Christman CW, Levasseur JE, Poulishock JT, Ellis EF: Free oxygen radicals in cerebral vascular responses. The Physiologist 26:165-169, 1983.

103. Lansman JB, Hallman TJ, Rink TJ: Single stretch-activated ion channels in vascular endothelial cells as mechano-transducers. Nature 325:811-813, 1987.

104. Lewis PJ, Weir BKA, Nosko MG, Tanabe T, Grace MG: Intrathec nimodipine therapy in a primate model of chronic cerebral vasospasm. Neurosurgery 22:492-500, 1988.

105. Lewis RA, Austen KF: The biologically active leukotrienes, biosynthesis, metabolism, receptors, functions and pharmacology. J Clin Invest 73:889-897, 1984.

106. Liszczak TM, Varsos VG, Black PMcL, et al: Cerebral arterial constriction after experimental subarachnoid hemorrhage is associated with blood components within the arterial wall. J Neurosurg 58:18-26, 1983.

107. Locksley HB: Report on the cooperative study of intracranial aneurysms and subarachnoid hemorrhage. Section V, Part II. Natural history of subarachnoid hemorrhage, intracranial aneurysms and arteriovenous malformations. Based on 6368 cases in the Cooperative Study. J Neurosurg 25:321-368, 1966.

108. Maroon JC, Nelson PB: Hypovolemia in patients with subarachnoid hemorrhage: therapeutic implications. Neurosurgery 4:223-225, 1979.

109. Marshal LF, Bruce DA, Bruno L, Langfitt TW: Vertebrobasilar spasm. A significant cause of neurological deficit in head injury. J Neurosurg 48:560-564, 1978.

110. Martin W, Villani GM, Jothianandan D, Furchgott RF: Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. J Pharmacol Exp Ther 232:708-716, 1985.

111. Martin W, Furchgott RF, Villani GM, et al: Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. J Pharmacol Exp Ther 237:529-538, 1986.

112. Maspes PE, Marini G: Intracranial arterial spasm related to supraclinoid ruptured aneurysms. Acta Neurochir (Wein) 10:630-638, 1962.

113. Matsuzaki T, Mizukami M, Kawase T, Tazawa T: Angiographic vasospasm and regional cerebral blood flow in the cases of ruptured aneurysm - correlation between vasospasm in the middle cerebral artery and delayed ischemic hemiparesis. Neurol Surg 9:1511-1516, 1981.

114. McCord JM: Oxygen-derived free radicals in post ischemic tissue injury. NEJM 312:159-163, 1985.

115. Mickel HS, Vaishnav YN, Kempski O, von Lubitz D, Weiss JF, Feuerstein G: Breathing 100% oxygen after global brain ischemia in mongolian gerbils results in increased lipid peroxidation and increased mortality. Stroke 18:426-430, 1987.

116. Mikawa T, Nonomura Y, Ebashi S: Does phosphorylation of myosin light chain have a direct relation to regulation in smooth muscle? J Biochem (Tokyo) 82: 1789-1791, 1977.

117. Misra HP, Fridovich I: The generation of superoxide radical during autoxidation of hemoglobin. J Biol Chem 247:6960-6962, 1972.

118. Mizoi K, Suzuki J, Imaizumi S, Yoshimoto T: Development of new cerebral protective agents: the free radical scavengers. Neurol Res 8:75-80, 1986.

119. Mizukami M, Usami T, Tazawa T, Kawase T: Prevention of vasospasm by removal of subarachnoid blood in early operation. Neurol Med Chir 21:1069-1077, 1981.

120. Moncada S, Palmer RM, Gryglewski RJ: Mechanism of action of some inhibitors of endothelium-derived relaxing factor. Proc Natl Acad Sci 83:9164-9168, 1986.

121. Morimoto K, Tagawa K, Hayakawa T, Watanabe F, Mogami H: Cellular level of purine compounds in ischemic gerbil brain by high performance liquid chromatography. J Neurochem 38:833-835,1982.

122. Muller A, Ghiglieri-Bertez C, Modat G, Bonne C: Specific binding of leukotriene C_4 to endothelial cell membranes. Prostaglandins Leukotrienes and Medicine 26:233-240, 1987.

123. Murad F, Miltal CK, Arnold WP, Katsuki S, Kimura H: Gyanylate cyclase: activation by azide, nitro compounds, nitric oxide and hydroxy radical and inhibition by hemoglobin and myoglobin. Adv Cyclic Nucleotide Res 9:145-158, 1978.

124. Nakagomi T, Kassell NF, Sasaki T, Fujiwara S, Lehman RM, Johshita H, Nazar GB, Torner JC: Effect of subarachnoid hemorrhage on endothelium-dependent vasodilation. J Neurosurg 66:915-923, 1987.

125. Nakagomi T, Kassell NF, Sasaki T, Fujiwara S, Lehman RM, Johshita H, Torner JC: Effects of hypoxia on the contractile response to KCl prostaglandin $F_{2\alpha}$, and hemoglobin. J. Neurosurg 67:565-572, 1987.

126. Nakagomi T, Kassell NF, Sasaki T, Fujiwara S, Lehman RM, Torner JC: Impairment of endothelium-dependent vasodilation induced by acetylcholine and adenosine triphosphate following experimental subarachnoid hemorrhage. Stroke 18:482-489, 1987.

127. Nakagomi T, Kassell NF, Sasaki T, Lehman RM, Torner JC, Hongo K, Lee JH: Effect of removal of the endothelium on

vasocontraction in canine and rabbit basilar arteries. J Neurosurg 68:757-766, 1988.

128. Nakayama K, Hashimota K: Blood components and cerebral vasospasm. Biblthca Cardiol 38:148-160, 1984.

129. Nosko M, Weir B, Krueger C, Cook D, Norris S, Overton T, Boisvert D: Nimodipine and chronic vasospasm in monkeys: Part 1, Clinical and radiological findings. Neurosurgery 16:129-136, 1985.

130. Nosko M, Weir BKA, Lunt A, Grace M, Allen P, Mielke B: Effect of clot removal at 24 hours on chronic vasospasm after SAH in the primate model. J Neurosurg 66:416-422, 1987.

131. Pakarinen S: Incidence, aetiology, and prognosis of primary subarachnoid hemorrhage. A study based on 589 cases diagnosed in a defined urban population during a defined period. Acta Neurol Scand 43 (Suppl 29): 1-28, 1967.

132. Palmer RMJ, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524-526, 1987.

133. Parker CW: Leukotrienes and prostaglandins in the immune system, in Zor et al (eds): *Advances in Prostaglandin, Thromboxane and Leukotriene Research*. New York: Raven Press, 1986, Vol 16, pp 113-134.

134. Parks DA, Bulkley GB, Granger DN: Role of oxygen-derived free radicals in digestive tract diseases. Surgery 94:415-422, 1983.

135. Parks DA, Granger DN: Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. Am J Physiol 245:9285-9289, 1983.

136. Peach MJ, Loeb AL, Singer HA, Saye J: Endothelium-derived vascular relaxing factor. Hypertension 7 (Suppl I): 194-1100, 1985.

137. Peach MJ, Singer HA, Izzo NJ, Loeb AL: Role of calcium in endothelium-dependent relaxation of arterial smooth muscle. Am J Cardiol 59:35A-43A, 1987.

138. Petruk KC, West M, Mohr G, et al: Nimodipine treatment in poor grade aneurysm patients, results of a multicenter, double-blind, placebo-controlled trial. J Neurosurg 68:505-517, 1988.

139. Phillips LH, Whisnant JP, O'Fallon WM, Sundt TM: The unchanging pattern of subarachnoid hemorrhage in a community. Neurology 30:1034-1040, 1980.

140. Pickard JD, Graham DI, Matear E, Macpherson P, Tamura A, Fitch W: Ultrastructure of cerebral arteries following experimental subarachnoid hemorrhage. J Neurol Neurosurg Psychiatry 48:256-262, 1985.

141. Piper PJ: Formation and action of leukotrienes. Physiol Rev 64:744-761, 1984.

142. Pitts LH, MacPherson P, Wyper DJ, Jennett WB: Cerebral blood flow, angiographic cerebral vasospasm, and subarachnoid hemorrhage. Acta Neurol Scan 56:334-335, 1977.

143. Pool JL: Early treatment of ruptured intracranial aneurysms of the circle of Willis with special clip technique. Bull NY Acad Med 35:357-369, 1959.

144. Rapoport RM, Draznin MB, Murad F: Endothelium-dependent vascular relaxation may be mediated through cyclic GMP-dependent protein phosphorylation. Clin Res 31:526A, 1983 (Abstract).

145. Ropper AH, Zervas NT: Outcome 1 year after SAH from cerebral aneurysm: management morbidity, mortality, and functional status in 112 consecutive good-risk patients. J Neurosurg 60:909-915, 1984.

146. Rosenblum WI: Endothelial dependent relaxation demonstrated in vivo in cerebral arterioles. Stroke 17:494-497, 1986.

147. Sakaki S, Kuwabara H, Ohta S: Biological defense mechanisms in the pathogenesis of prolonged cerebral vasospasm in the patients with ruptured intracranial aneurysms. Stroke 17:196-201, 1986.

148. Sano K, Asano T, Tanishima T, Sasaki T: Lipid peroxidation as a cause of cerebral vasospasm. Neurol Res 2:253-272, 1980.

149. Sano K: Cerebral vasospasm and aneurysm surgery. Clin Neurosurg 30:13-58, 1982.

150. Sano K: Timing of surgery: the Japanese experience, in Battye R (ed): *Calcium Antagonists: Possible Therapeutic Use in Neurosurgery*. New York: Raven Health Care Communication, 1983, pp 35-42.

151. Sano K: Timing of surgery, operative mortality, and followup results in cases with subarachnoid hemorrhage due to aneurysm rupture, in Auer LM (ed): *Timing of Aneurysm Surgery*. Berlin: Walter de Gruyter, 1985, pp 227-236.

152. Sasaki T, Kassell NF, Yamashita M, Fujiwara S, Zuccarello M: Barrier disruption in the major cerebral arteries following experimental subarachnoid hemorrhage. J Neurosurg 63:433-440, 1985.

153. Sasaki T, Mayanagi Y, Yano H, Kim S: Cerebral vasospasm with subarachnoid hemorrhage from cerebral arteriovenous malformation. Surg Neurol 16:183-187, 1981.

154. Sasaki T, Murota S-I, Wakai S, Asano T, Sano K: Evaluation of prostaglandin biosynthetic activity in canine basilar artery following subarachnoid injection of blood. J Neurosurg 55:771-778, 1981.

155. Sasaki T, Tanishima T, Asano T, Nayanagi Y, Sano K: Significance of lipid peroxidation in the genesis of chronic vasospasm following rupture of an intracranial aneurysm. Acta Neurochir 28 (Suppl): 536-540, 1979.

156. Sasaki T, Wakai S, Asano T, Watanabe T, Kirino T, Sano K: The effect of lipid hydroperoxide of arachidonic acid on the canine basilar artery, an experimental study on cerebral vasospasm. J Neurosurg 54:357-365, 1981.

157. Shiobara R, Kawase T, Toya S, Ebato K, Miyahara Y: "Scavenger Surgery" for subarachnoid hemorrhage, continuous ventriculo-cisternal perfusion using artificial cerebrospinal fluid with urokinase, in Auer L (ed): *Timing of Aneurysm Surgery*. Berlin: de Gruyter, 1985.

158. Shlafer M, Kane PF, Wiggins VY, Kirsh MM: Possible role for cytotoxic oxygen metabolites in the pathogenesis of cardiac ischemic injury. Circulation 66 (Suppl): I85-I92, 1982.

159. Siesjo BK: Cerebral Circulation and Metabolism. J Neurosurg 60:883-908, 1984.

160. Smith RR, Yoshioka J: Intracranial arterial spasm, in Wilkins RH, Rengachary SS (eds): *Neurosurgery*. New York: McGraw-Hill, 1985, Vol 2, pp 1355-1362.

161. Smith RR: Pathophysiology and clinical evaluation of subarachnoid hemorrhage, in Youmans JR (ed): *Neurological Surgery. Development and Acquired Anomalies: Vascular Disease.* Toronto: Saunders, 1982, Vol 3, pp 1627-1644.

162. Smith RR, Clower BR, Grotendorst GM, Yabuno N, Cruse JM: Arterial wall changes in early human vasospasm. Neurosurgery 16: 171-176, 1985.

163. Solomon RA, Post KD, McMurtry III JG: Depression of circulating blood volume in patients after subarachnoid hemorrhage: implications for the management of symptomatic vasospasm. Neurosurgery 15:354-361, 1984.

164. Sonobe M, Suzuki J: Vasospasmogenic substance produced following subarachnoid hemorrhage, and its fate. Acta Neurochir 44:97-106, 1978.

165. Sutton HC, Roberts PB, Winterbourn CC: The rate of reaction of superoxide radical ion with oxyhaemoglobin and meth-haemoglobin. Biochem J 155:503-510, 1976.

166. Suttorp N, Toepfer W, Roka L: Antioxidant defense mechanisms of endothelial cells: glutathione redox cycle versus catalase. Am J Physiol 251:C671-C680, 1986.

167. Suzuki J, Imaizumi S, Kayama T, Yoshimoto T: Chemiluminescence in hypoxic brain - the second report: cerebral protective effect of mannitol, Vitamin E and glucocorticoid. Stroke 16:695-700, 1985.

168. Suzuki J, Komatsu S, Sato T, et al: Correlation between CT findings and subsequent development of cerebral infarction due to vasospasm in subarachnoid hemorrhage. Acta Neurochir 55:63-70, 1980.

169. Suzuki N, Nakamura T, Imabayashi S, Ishikawa Y, Sasaki T, Asano T: Identification of 5-hydroxy eicosatetraenoic acid in cerebrospinal fluid after subarachnoid hemorrhage. J Neurochem 41:1186-1189, 1983.

170. Symon L, Ackerman R, Ball JWD, DuBoulay EPGH, Marshal J, Rees JE, Russell RWR: The use of xenon clearance method in subarachnoid hemorrhage: post-operative studies with clinical and angiographic correlation. Eur Neurol 8:8-14, 1972.

171. Symon L, Bell BA, Kendall B: Relationship between effused blood and clinical course and prognosis in aneurysmal subarachnoid hemorrhage: a preliminary computerized tomography scan study, in Wilkins RH (ed): *Cerebral Arterial Spasm*. Baltimore: Williams and Wilkins, 1980, pp 409-411.

172. Takahashi S, Sonobe M, Nagamira Y: Early operations for ruptured intracranial aneurysms. Comparative study with computed tomography. Acta Neurochir 57:23-31, 1981.

173. Tanabe T, Saitoh T, Tachibana S, Takagi H, Yada K: Effect of hyperdynamic therapy on cerebral ischemia caused by vasospasm associated with subarachnoid hemorrhage. Acta Neurochir 63:291-296, 1982.

174. Taneda M: Effects of early operation for ruptured aneurysms on prevention of delayed ischemic symptoms. J Neurosurg 57:622-628, 1982.

175. Tappel AL: The mechanism of the oxidation of unsaturated fatty acids catalyzed by hematin compounds. Arch Biochem Biophys 44:378-395, 1953.

176. Taylor AE, Martin D, Parker JC: The effects of oxygen radicals on pulmonary edema formation. Surgery 94:433-438, 1983.

177. Toda N, Shimizu K, Ohta T: Mechanism of cerebral arterial contraction induced by blood constituents. J Neurosurg 53:312-322, 1980.

178. Travis MA, Yonkers PA, Hall ED: Attenuation of post-hemorrhagic and post-ischemic cerebral hypoperfusion by the 21-aminosteroid U-74006F, a potent inhibitor of iron-dependent lipid peroxidation. Neuroscience 13:1494, 1987 (abstract).

179. Van Benthuysen KM, McMurtry IF, Horwitz LD: Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. J Clin Invest 79:265-274, 1987.

180. Vanhoutte PM, Houston DS: Platelets, endothelium, and vasospasm. Circulation 72:728-734, 1985.

181. Vanhoutte PM: Could the absence or malfunction of vascular endothelium precipitate the occurrence of vasospasm? J Mol Cell Cardiol 18:679-689, 1986.

182. Van Neuter JM: Vascular pharmacology of calcium entry blockers, in Godfraind T, Herman AG, Willens D (eds): *Calcium Entry Blockers in Cardiovascular and Cerebral Dysfunction*. Boston: Martines Nijhoff, 1984, pp 59-72.

183. Vinall PE, Simeone FA: Effects of oxygen and glucose deprivation on vasoactivity in isolated bovine middle cerebral arteries. Stroke 17:970-975, 1986.

184. Wahl M: A review of neurotransmitters and hormones implicated in mediating cerebral vasospasm, in Voth D, Glees P (eds): *Cerebral Vascular Spasm*. Berlin: Walter de Gruyter, 1985, pp 224-230.

185. Wei EP, Kontos HA, Dietrich WD, Povlishock JT, Ellis EF: Inhibition by free radical scavengers and cyclooxygenase inhibitors of pial arteriolar abnormalities from concussive brain injury in cats. Circ Res 48:95-103, 1981.

186. Weir B: Intracranial aneurysms and subarachnoid hemorrhage: an overview, in Wilkins RH, Rengachary SS (eds): *Neurosurgery*. New York: McGraw-Hill, 1985, Vol 2, pp 1308-1329.

187. Weir B: *Aneurysms Affecting the Nervous System*. Baltimore: Williams and Wilkins, 1987.

188. Weir B, Aronyk K: Management mortality and the timing of surgery for supratentorial aneurysms. J Neurosurg 54:146-150, 1981.

189. Weir B, Grace M, Hansen J, Rothberg C: Time course of vasospasm in man. J Neurosurg 48:173-178, 1978.

190. Weir BK: Pathophysiology of vasospasm. Int Anaes Clin 20:39-43, 1982.

191. Weir BKA: The relationship between arterial spasm and neurological deficits, in Battye R (ed): *Calcium Antagonists: Possible Therapeutic Use in Neurosurgery*. New York: Raven Health Care Communications, 1983, pp 45-52.

192. Weir B, Menon D, Overton T: Regional cerebral blood flow inpatients with aneurysms: estimation by xenon 133 inhalation. Can J Neurol Sci 5:301-305, 1978.

193. Wellum GR, Irvine TW, Zervas NT: Cerebral vasoreactivity of home proteins in vitro, some mechanistic considerations. J Neurosurg 56:777-783, 1982.

194. Welsh FA, Ginsberg MD, Rieder W, Budd WW: Diffuse cerebral ischemia in the cat: II. Regional metabolites during severe ischemia and recirculation. Ann Neurol 3:493-501, 1978.

195. White BC, Krause GS, Aust SD, Eyster GE, Lansing E: Post ischemic tissue injury by iron-mediated free radical lipid peroxidation. Ann Emerg Med 14:804-809, 1985.

196. Wilkins RH: Attempts at prevention or treatment of intracranial arterial spasm: an update. Neurosurgery 18:808-825, 1986.

197. Wilkins RH, Odom GL: Intracranial arterial spasm associated with craniocerebral trauma. J Neurosurg 32:626-633, 1970.

198. Wilson RB, Middleton CC, Sun GY: Vitamin E, antioxidants and lipid peroxidation in experimental atherosclerosis of rabbits. J Natr 108:1858-1867, 1978.

199. Winterbourn CC, McGrath BM, Carrell RW: Reaction involving superoxide and normal and unstable hemoglobin. Biochem J 155:493-502, 1976.

200. Wood JH, Fleischer AS: Observations during hypervolemic hemodilution of patients with acute focal cerebral ischemia. JAMA 248:2999-3004, 1982.

201. Yamashima T, Yamamoto S: Cerebral arterial pathology in experimental subarachnoid hemorrhage. J Neurosurg 58:843-850, 1983.

202. Yoshida S, Inoh S, Asano T, Sano K, Kubota M, Shimazaki H, Ueta N: Effect of transient ischemia on free fatty acids and phospholipids in the gerbil brain: lipid peroxidation as a possible cause of post-ischemic injury. J Neurosurg 53:323-331, 1980.

203. Yoshida S, Abe K, Busto R, Watson BD, Kogure K, Ginsberg MD: Influence of transient ischemia on lipid-soluble antioxidants, free fatty acids and energy metabolites in rat brain. Brain Res 245:307-316, 1982.

204. Yoshida Y, Ueki S, Takahashi A, Takagi H, Torigoe H, Kudo S: Intrathecal irrigation with urokinase in ruptured cerebral aneurysm cases. Neurol Med Chir (Tokyo) 25:989-997, 1985.

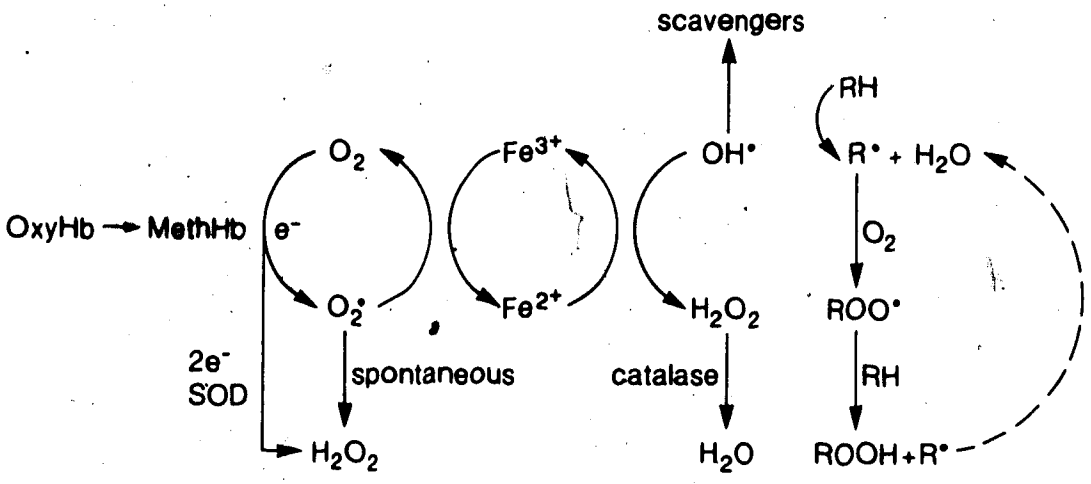
205. Zaleska MM, Floyd RA: Regional lipid peroxidation in rat brain in vitro: possible role of endogenous iron. Neurochem Res 10:397-410, 1985.

206. Zervas NT, Liszczak TM, Mayberg MR, et al: Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. J Neurosurg 56:475-481, 1982.

207. Zingesser LH, Schechter MM, Dexter J, Katzman R, Scheinberg LC: On the significance of spasm associated with rupture of cerebral aneurysm: the relationship between spasm as noted angiographically and regional cerebral blood flow determinations. Arch Neurol 18:520-528, 1968.

APPENDIX A

Iron Dependent Lipid Peroxidation



OxyHb, oxyhemoglobin; MethHb, meth-hemoglobin, SOD, superoxide dismutase; Fe, ferrous and ferric iron; $O_2 \bullet$, superoxide radical; $OH \bullet$, hydroxy radical; $R \bullet$, alkyl radical; $ROO \bullet$, lipid peroxy radical. (Modified from Del Maestro RF: An approach to free radicals in medicine and biology. Acta Physiol Scand, Suppl 492:153-68, 1980.)

PUBLICATIONS

Steinke D, Weir B, Disney L: Hydrocephalus following aneurysmal subarachnoid haemorrhage. Neurol Research 9:3-9, 1987.

Findlay JM, Weir B, Steinke D, Tanabe T, Gordon P, Grace M: The effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic cerebral vasospasm in a primate model of subarachnoid hemorrhage. J Neurosurg, 1988. (In Press.)

Steinke DE, Weir BKA, Findlay JM, Tanabe T, Grace M, Krushelnycky BD: A trial of the 21-aminosteroid U74006F in a primate model of chronic cerebral vasospasm. (Submitted for publication.)

Steinke DE, Weir BKA, Krueger C, Cook D, Grace M: Trial of U74006F in a primate model of chronic cerebral vasospasm: Studies of endothelium dependent relaxation. (Submitted for publication.)